SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME 3-(PIPERAZIN-1-YL)CINNOLINE DERIVATIVES

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Committee Decision

This thesis (Synthesis, characterization and biological activity of some 3-(piperazin-1-yl)cinnoline derivatives) was successfully defended and approved on Wednesday 27-04-2011

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I dedicate this work to my lovely family (Parents, brother and sisters)

To my wonderful husband and my sweet daughter.

Acknowledgements

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Love and thanks are due to my parents who taught me the value of education, and to all of my family members. I am deeply indebted to them for their continued support and unwavering faith in me to reach this stage.

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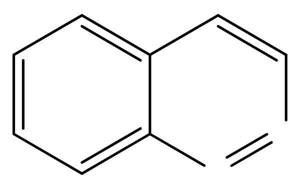
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Abstract

A new series of 6-substituted-4-methyl-3-(4-aryl-piperazin-1-yl)cinnolines (**41-43**) were synthesized as potential antibacterial and antifungal agents *via* intramolecular cyclization of piperazinyl amidrazone derivatives **(38-40)** catalyzed by polyphosphoric acid (PPA). Compounds **38-40** were synthesized through direct interaction of the hydrazonyl chloride (**37a-d**) with the corresponding *N*-substituted piperazine in the presence of triethylamine. The new compounds were characterized by different spectroscopic techniques, such as ^{1}H and ^{13}C-NMR, DEPT and mass spectrometry.

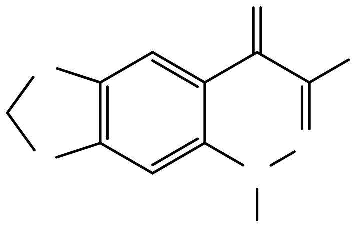
1. INTRODUCTION

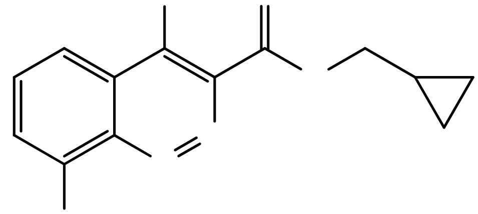
Benzopyridazine nucleus or 1,2-diazanaphtalene (**1**), well-known as cinnoline and its derivatives have received considerable interest due to their wide range of pharmacological profiles, e.g. antibacterial (Barraja *et al*., 1999), antitumor (Hennequin *et al*., 1999; Yu *et al*., 2003), antifungal (Nargund *et al*., 1992) and antiinflammatory (Bantick *et al*., 1998) activities. Compounds of the cinnoline series have antithrombocytic (Rehse and Gonska, 2005) and antituberculosis (Ramalingam *et al*., 2006) characteristics, and also exhibit anesthetizing (Gomtsyan *et al*., 2005), and sedative (Alvarado *et al*., 2006) activity in addition to their use as agrochemicals (Abdelrazek *et al*., 2006).



Significant commercial interest in the development of benzopyridazine derivatives, particularly pharmaceutical uses of pyridazines and cinnolines, is shown by the large number of patents filed in this area (Heinisch and Frank, 1990). However, their ring system is an isosteric relative to either quinoline or isoquinoline. Therefore, in many cases the synthesized compounds were designed as analogs of the previously obtained quinoline or isoquinoline derivatives; for example cinoxacine **(2)** is a cinnoline analogue of quinoline antibacterials used for urinary tract infection (Coates, 1996) and ICI-D-7569 **(3)** is an

anxiolytic agent (Melikian *et al*., 1992). Meanwhile, attention has been paid to the synthesis of heterocyclic compounds bearing a cinnoline moiety.

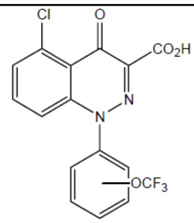
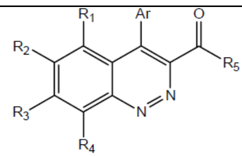
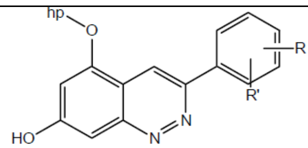
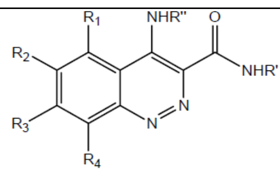
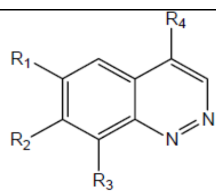
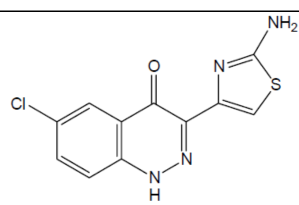
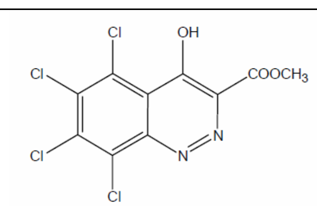




Compounds containing a cinnoline moiety exhibit a series of interesting physical characteristics. Thus, luminescence was detected in pyrrolo[1,2-*b*]cinnolines (Mitsumori *et al*., 2003). Furthermore, aryl-substituted cinnolines were used as materials for nonlinear optics (Chapoulaud *et al*., 2000; Busch *et al*., 2007). The structures of some selected cinnolines and their biological activities are shown in Table 1.

Cinoxacine (2)

Table 1. Selected cinnoline derivatives and their biological activities.

No.	Structure	Activity	Reference
(4)		Herbicide	Toyonaka *et al.*, 1989
(5)		Modulators of liver X receptors (LXRS)	Hu *et al*., 2006
(6)		Antitumor	Kuroiwa *et al.*, 2005
(7)		Dementia due Alzheimer's disease	Chapdelaine *et al.*, 2007
(8)		PDE10	Hitchcock *et al.*, 2007
(9)		Antibacterial activity	Vingkar *et al.*, 2001
(10)		Antiallergic activity	Gilman, 1972

1.1. Synthesis of cinnoline derivatives

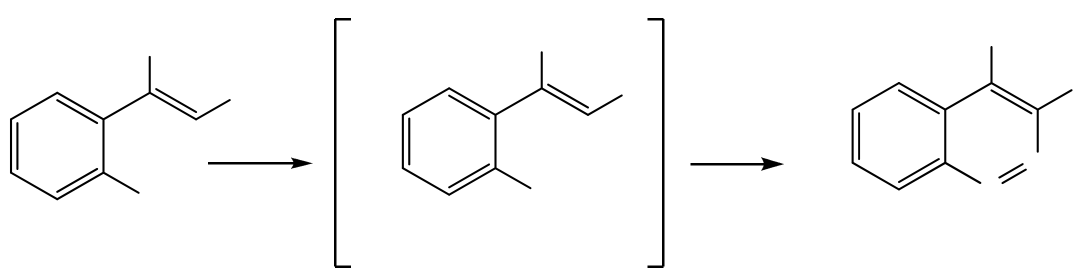
Different methods were described for the preparation of cinnoline derivatives as potential pharmaceuticals with different substituents. However, the main synthetic route to cinnolines is the cyclization of *o*-substituted arylhydrazones or diazotization of *o*-substituted anilines. Both methods have been widely adopted as preparative approaches for the synthesis of cinnoline moiety.

The following methods are described briefly for the preparation of cinnolines:

1.1.1. *via* Diazonium salts:

1.1.1.1. The Widman-Stoermer synthesis

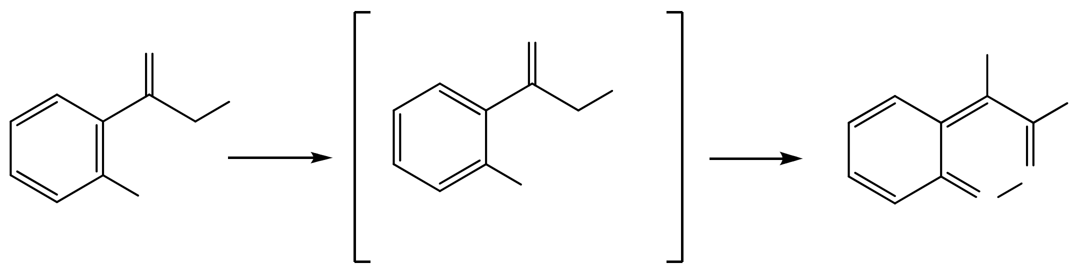
Widman–Stoermer synthesis is one of the most widely used methods for the preparation of 3-substituted or 3,4-disubstituted cinnolines (**12** / Scheme 1) by cyclization of diazotized 2-(alken-1-yl)anilines (**11**) (Nunn *et al.,*1953; Haddlesey *et al.*,1964). The product was achieved in good yield in presence of electron-donating R_1 groups, but was retarded when R_1 = hydrogen or a carboxy group.



Scheme 1. Cyclization of diazotized 2-(alken-1-yl)aniline derivatives.

1.1.1.2. The Borsche synthesis

The most versatile and widely employed route to cinnoline synthesis is the Borsche synthesis. This method describes the synthesis of 4-hydroxy-cinnolins (**14**) by diazotization of 2-acylanilines (**13**), as in the case of 2-acetylanilines and 2-(haloacetyl)anilines (Scheme 2). The cyclization is carried out at room temperature, but the reaction proceedes slowly, although it can be accelerated either by electron-withdrawing groups on *α*-position to the acetyl moiety, or by carrying out the reaction at higher temperatures.



Scheme 2. Synthesis of 4-hydroxycinnoline derivatives *via* Borsche synthesis.

A more recent variation of the Borsche approach is the cyclization of triphenylphosphoranylidene compounds (**15**) to 4-cinnolinones (**16**), which can be subsequently hydrolyzed to give 4-hydroxycinnoline derivatives (**17** / Scheme 3) (Baldoli *et al.*, 1987).

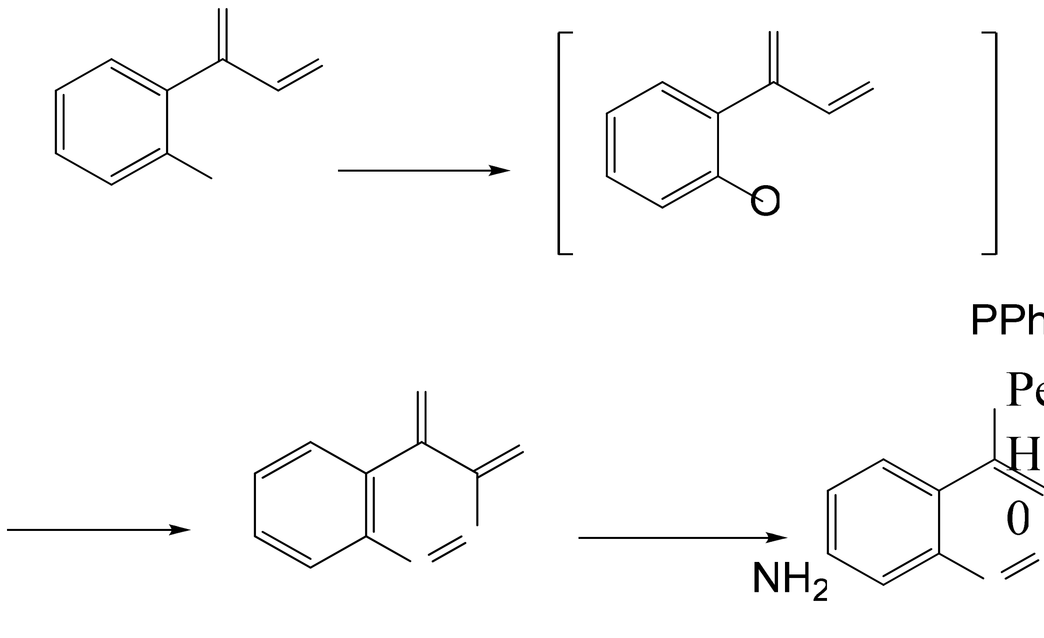
O

R

HNO_2

NH_2

13



Scheme 3. Modified method of Borsche synthesis using triphenylphosphoranylidene compounds.

15

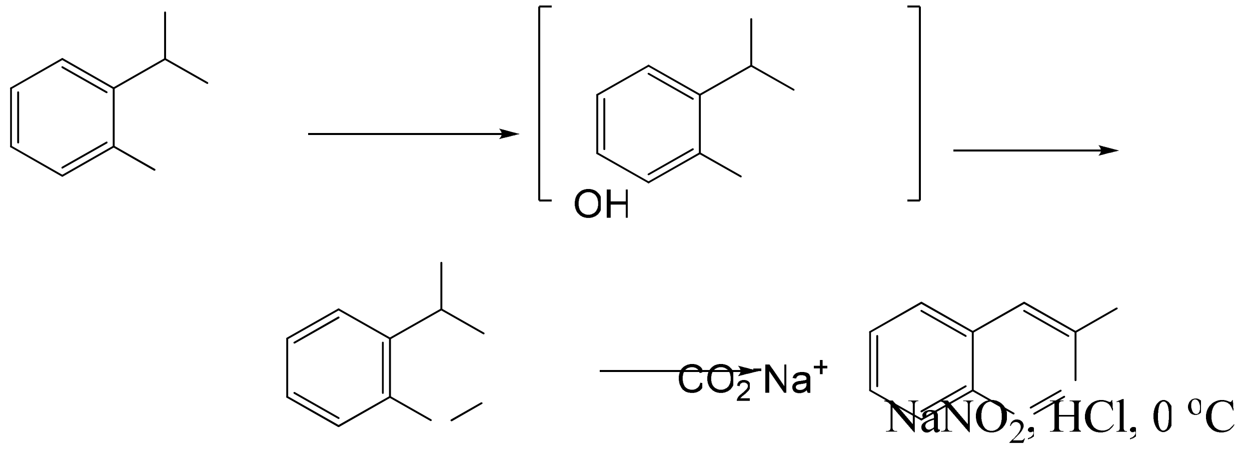
1.1.1.3. The Neber–Bossel synthesis

The Neber–Bossel synthesis is performed by diazotization of 2-(2-aminophenyl)-2-hydroxyacetate derivatives (**18**) and subsequent reduction of the resulting diazonium salt with tin(II) chloride to yield the respective 2-(2-hydrazinophenyl)-2-hydroxyacetic acids (**19**). The latter, in turn, undergoe cyclization in boiling aqueous hydrochloric acid to give 3-hydroxycinnoline derivatives (**20** / Scheme 4) (Neber *et al.*, 1929; Zey, 1972; Lora-Tamayo *et al.*, 1976).

10 % aq. NaOH

68-91%

16

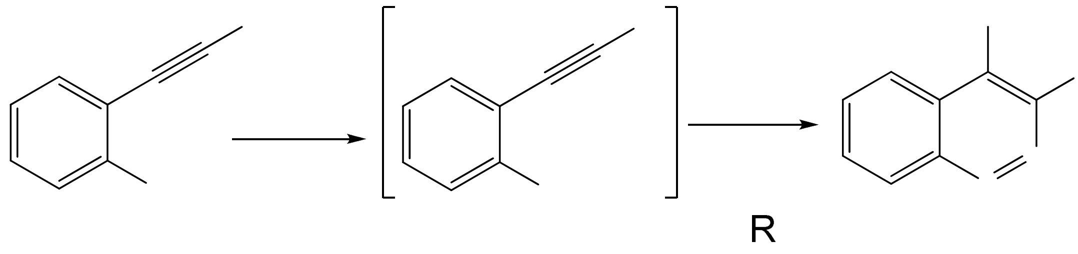


Scheme 4. Synthesis of 3-hydroxycinnoline derivatives *via* Neber-Bossel approach.

1.1.1.4. The Richter synthesis

A general approach for the synthesis of 3-substituted 4-hydroxycinnoline derivatives (Schofield and Simpson, 1945) *via* thermal cyclization of 2-alkylbenzenediazonium salts is known as the Richter synthesis (von Richter, 1883).

4-Hydroxycinnolines were prepared by cyclization of compound (**21**) to afford 4-halocinnolines (**22**, X = Cl, Br) using concentrated hydrochloric or hydrobromic acid, respectively, in the diazotization reaction and keeping the temperature between 25 and 30 °C (Scheme 5). The 4-hydroxy group is formed by hydrolysis of the intermediate 4-chloro or 4-bromo derivatives (Vasilevsky *et al.*, 1994; Vasilevsky and Tretyakov, 1995).



Scheme 5. Synthesis of 4-halocinnoline derivatives *via* Richter synthesis.

1.1.2. *via* Phenylhydrazones:

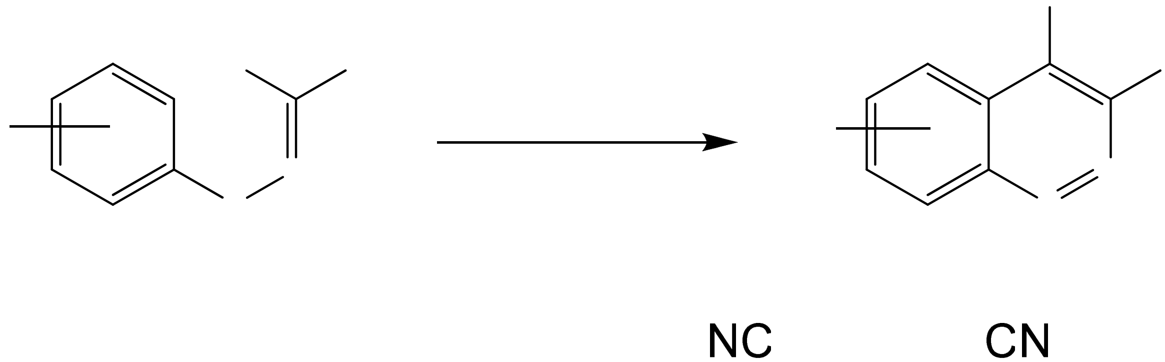
1.1.2.1. Oxomalonic acid derivatives

These compounds can be obtained by intramolecular cyclization *via* Friedel-Crafts reaction of (phenylhydrazono) malonoyl dichloride (**23**) catalyzed with titanium(IV) chloride as Lewis acid to yield 4-hydroxycinnoline-3-carboxylic acid derivatives (**24** / Scheme 6). The yield is markedly reduced in presence of deactivating groups in the 2- or 4-positions (Barber *et al.*, 1961).



Scheme 6. Cyclization of arylhydrazonomalonic acid chlorides.

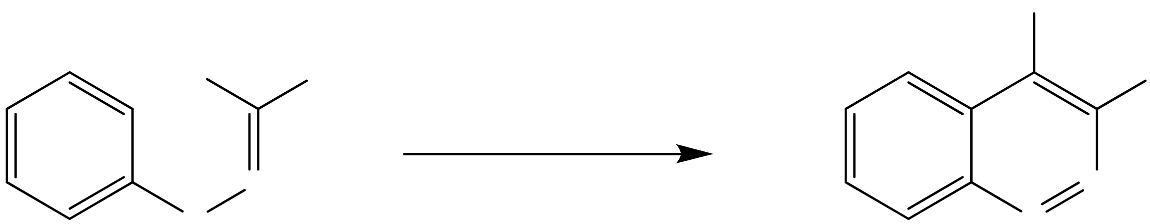
In a related reaction, intracyclization of (phenylhydrazono)malononitrile (**25**) with aluminum trichloride in benzene gave 4-aminocinnoline-3-carbonitrile (**26** / Scheme 7) (Gewald *et al.*, 1984).



Scheme 7. Cyclization of (arylhydrazono)malononitrile derivatives.

1.1.2.2. Cyclization of aryl trifluoromethyl ketone phenylhydrazone derivatives

The syntheses of 3-arylcinnolin-4-amine derivatives (**28**) were achieved in good yields by cyclization of the appropriate aryl trifluoromethyl ketone derivatives (**27**) in presence of potassium hexamethyldisilazanide (KHMDS) (Kiselyov, 1995). This reaction offers an easy access to prepare different aryl substituents in the 3-position by variation of the aryl group in the aryl trifluoromethyl ketone (Scheme 8).



Scheme 8. Preparation of 3-arylcinnolin-4-amine derivatives *via* cyclization of aryl trifluoromethyl ketone phenylhydrazone derivatives.

1.1.2.3. Synthesis of 3-aroylcinnolines from arylhydrazono-3-oxopropanal derivatives

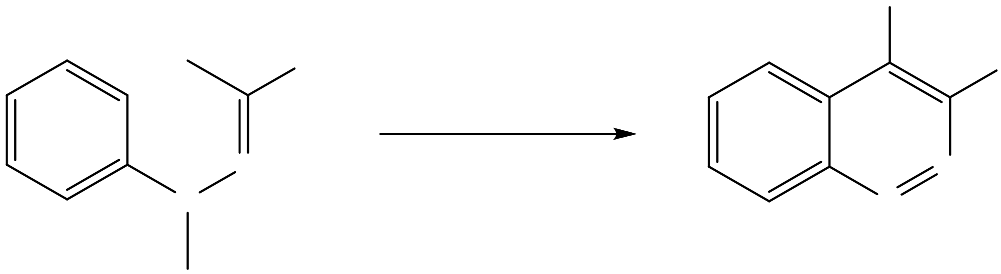
Acid-catalyzed cyclization of 3-aryl-2-(arylhydrazono)-3-oxopropanal derivatives (**29**) in concentrated sulfuric or polyphosphoric acid afforded the corresponding 3-aroylcinnolines (**30** / Scheme 9) (Al-Awadi *et al.*, 2001).



Scheme 9. Synthesis of 3-aroylcinnolines from 3-aryl-2-(arylhydrazono)-3-oxopropanal derivatives.

1.1.2.4. Synthesis of 3-arylcinnoline-4-carbonitriles by reaction of acetophenone phenylhydrazones

The reaction of substituted acetophenone (methyl or phenyl)hydrazones (**31**) and tetracyanoethene (TCNE) leads to 3-arylcinnoline-4-carbonitriles (**32** / Scheme 10) (Matsubara *et al.*, 1997). The mechanism of this unusual one-step synthesis still remains a mystery.



Scheme 10. Synthesis of 3-arylcinnoline-4-carbonitrile derivatives by reaction of acetophenone methyl(phenyl)hydrazones and tetracyano ethene.

1.2. Reactivity of cinnoline and its physical prosperities

Cinnoline (**1**) is a pale yellow solid (Busch and Rast, 1897) which is soluble in water and organic solvents (Jacobs *et al*., 1946; Morley, 1951). It is a weak base and forms stable salts, such as the hydrochloride, the perchlorate (Ames *et al*., 1967) or the picrate (Busch and Rast, 1897; Jacobs *et al*., 1946).

Cinnoline derivatives, as well as 3- and 4-alkylcinnolines, react with simple electrophiles such as the nitronium ion (NO_2^+) to give mixtures of 5- and 8-substituted products (Moodie *et al*., 1968; Heinisch and Koch, 1983). Reduction of cinnoline (**1**) and its derivatives with lithium aluminum hydride leads to 1,4-dihydrocinnolines (Besford *et al*., 1963); the latter may rearrange to give indole derivatives under certain reaction conditions (Haddlesey *et al*.,1964). Thus, refluxing **1** with amalgamated zinc in aqueous acetic acid for four minutes resulted in the formation of 1,4-dihydrocinnoline, but after two hours at reflux indole was obtained (Besford and Bruce, 1964). Similarly, 3- methyl and 3-phenylcinnoline afford 3-methyl and 3-phenylindole, respectively (Bruce, 1959). Oxidation with peracids or hydrogen peroxide yields mixtures of cinnoline 1-oxides and 2-oxides

(Suzuki *et al*., 1966). Treatment of 4-phenylcinnoline with hot aqueous potassium permanganate causes cleavage of the benzene ring to give 5-phenylpyridazine-3,4-dicarboxylate (Stoermer and Fincke, 1909). The above experimental results show that electrophilic substitution occurs preferentially at carbon atoms in positions 5 and 8. Moreover, the electron density of nitrogen atom N-1 is slightly higher than N-2; however, the observed preferential attack at N-2 in N-oxidation, protonation, and alkylation reactions can be explained by steric hindrance for attack at N-1 by the peri C-8 proton (Palmer *et al*., 1971; Longuet-Higgins and Coulson, 1949).

1.3. ^{1}H & ^{13}C-NMR spectra data of cinnoline

The ^{1}H-NMR data of cinnoline and substituted cinnolines have been provided by several authors (Katritzky *et al*., 1967, Guy *et al*., 1978), whereas relatively little has been reported concerning ^{13}C-NMR data of these compounds (Pugmire *et al*., 1969; Dewar and Maitlis, 1957).

The ^{1}H-NMR data of cinnoline (**1**) in acetone-d_6 (Black and Heffernan, 1965) with the coupling constant (*J*) values and the ^{13}C-NMR chemical shifts in deuterated chloroform (Pugmire *et al*., 1969) are given in Fig. 1.

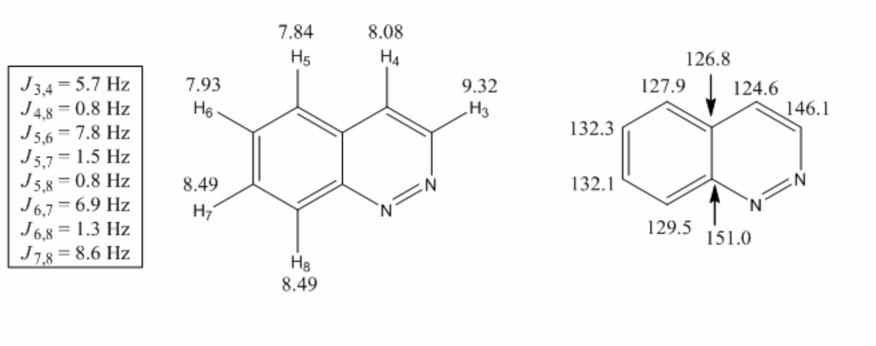
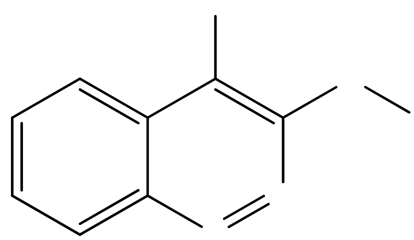


Fig. 1. ^{1}H and ^{13}C-NMR data of cinnoline.

2. Purpose of the Present Work

The aim of the present work is the synthesis of new cinnoline derivatives of potential antibacterial activity, since cinnolines have demonstrated interesting biological activities (Lewgowd and Stanczak, 2007). The chemistry and synthesis of cinnolines has been reviewed (Haider and Holzer, 2004; Vinogradova and Balova, 2008).

As far as we know, only a limited number of 3-(substituted)aminocinnolines have been reported (Aljarin *et al*., 2009; Bakathavachalam *et al*., 1984; Scobie and Tennant, 1994); examples include compounds (**33**) (Aljarin *et al*., 2009) (Fig. 4).

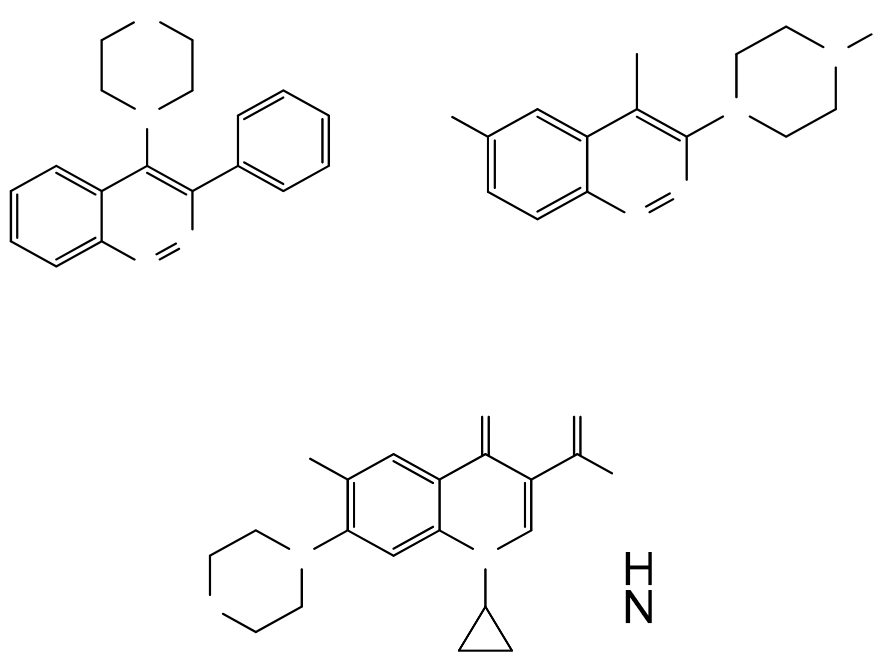


R= Ph; *p*-C_6H_4Cl; *p*-C_6H_4Me

Ar = *o, m, p*-C_6H_4Me; *p*-C_6H_4OMe; *o, p*-$C_6H_4(CHMe_2)$

To the best of our knowledge, only one patent dealt with cinnoline derivatives carrying (*N*-substituted)piperazines (**34**) as ring appendages attached to the pyridazine moiety at C-4; (Lowrie, 1966) (Fig. 5). Noteworthy, piperazine-containing compounds are of great interest due to their broad spectrum of biological activities (Lewgowd and

Stanczak, 2007). We envisaged that the combination of both moieties might enhance the biological activity, and this could be in parallel with the enhancement of the potency of the quinolone drugs containing piperazine species, such as ciprofloxacin (**35**) (Silva *et al.*, 1983) (Fig. 5).



Herein we saught to synthesize a selected series of amidrazones modified with some piperazine derivatives to yield the respective 1-(4-aryl-1-piperazinyl)-1-(phenylhydrazono)-2-propanones (**38-40**). Intramolecular cyclization of the latter compounds, using polyphosphoric acid, would give new 3-(4-aryl-1-piperazinyl)cinnoline derivatives (**41-43**). The structures of the proposed new compounds will be characterized by different spectroscopic techniques such as NMR and mass spectrometry.

In addition, the biological activities of some of the prepared compounds will be investigated. The synthetic strategy for the preparation of the target compounds is provided by a sequence of steps including:

1. Preparation of 1-chloro-1-(4-substitued-phenylhydrazono)-2-propanone (**37a-d)** *via* Japp-Klingemann reaction using aniline derivatives (**36a-d**) as starting materials which involves diazotizaion of the anilines and coupling of the resulting arenediazonium chlorides with 3-chloro-2,4-pentadione.

2. Synthesis of selected set of 1-(2-(4-substituted-phenyl)hydrazono)-1-(4-aryl-piperazinyl)-2-propanones (**38-40)** by the reaction of compounds **37a-d** with selected piperazine derivatives.

3. Cyclization of these intermediate amidrazone derivatives **(38-40)**, by polyphospheric acid, to afford the corresponding 6-substituted-4-methyl-3-(4-aryl-piperazinyl)cinnolines (**41-43** / Scheme 13).

All new target compounds (**41-43)** were characterized by different spectroscopic techniques such as NMR, mass spectrometry, and will be tested *in vitro* for their antibacterial activity.

3. Experimental

3.1. Materials and equipment

The following chemicals were used as received without further purification: substituted anilines and polyphosphoric acid (Fluka), 3-chloro-2,4-pentanedione, 1-(4-flourophenyl)piperazine, 1-(4-phenyl)piperazine, 1-(4-benzyl)piperazine (Janssen Chemica). The reactions were monitored by thin layer chromatography (TLC), carried out on 0.25 silica gel glass plates (60 F-254, Scharlau). Plates were visualized under UV light (where appropriate). Preparative thick layer chromatography was performed on 0.5 x 20 x 20 silica gel glass plates (60 F-254, Scharlau).

The ^{1}H- and ^{13}C-NMR spectra were recorded on a 300 MHz spectrometer (Bruker DPX-300) in $CDCl_3$ with *TMS* as an internal standard. The chemical shifts are reported in parts per million (ppm) expressed in δ units; coupling constant (*J*) values are given in Hertz (Hz). High resolution mass spectra (HRMS) were acquired using electrospray ionization (ESI) technique on a Bruker APEX-4 instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol / water 1:1 v/v + 0.1 % formic acid) and infused using a syringe pump with a flow rate of 2 μL / min. External calibration was conducted using arginine cluster in a mass range m/z 175-871. Melting points were recorded on SMP1 stuart apparatus and are uncorrected.

3.2. Synthesis

3.2.1. General procedure for the synthesis compounds (37a-d)

To a stirred solution of substituted aniline (8.1 mmol) in HCl (20 ml, 3 M) was dropwise added a cold solution of sodium nitrite (0.59 g, 8.6 mmol) at (0-5 ^{o}C) for 20-30 minutes. The prepared solution was added to a cold mixture of α-chloroacetylacetone (1.10 g, 8.1 mmol), sodium acetate (8.2 g, 100 mmol), and ethanol (200 mL) in one portion and the reaction mixture was stirred vigorously. The solid was filtered off, washed with water (5 x 100 mL), and crystallized from ethanol to afford the title compounds (Scheme 13).

The following compounds were prepared according to the above procedure:

*1-Phenylhydrazono-1-chloroacetone (**37a**)*, Yield (76%); M.p 138-140 °C (Lit. M.p 141-142 °C) (El-Abadelah *et al*., 1991).

*1-(p-Flourophenyl)hydrazono-1-chloroacetone (**37b**)*, Yield (71%); M.p 146-148 ^{o}C. (Lit. M.p 147-149 °C) (El-Abadelah *et al*., 1991).

*1-(p-Chlorophenyl)hydrazono-1-chloroacetone (**37c**)*, Yield (75%); M.p 176-177 °C (Lit. M.p 178-179 °C) (El-Abadelah *et al*., 1991).

*1-(p-Bromophenyl)hydrazono-1-chloroacetone (**37d**)*, Yield (74%); M.p 163-165°C (Lit. M.p 166-167 °C) (El-Abadelah *et al*., 1991).

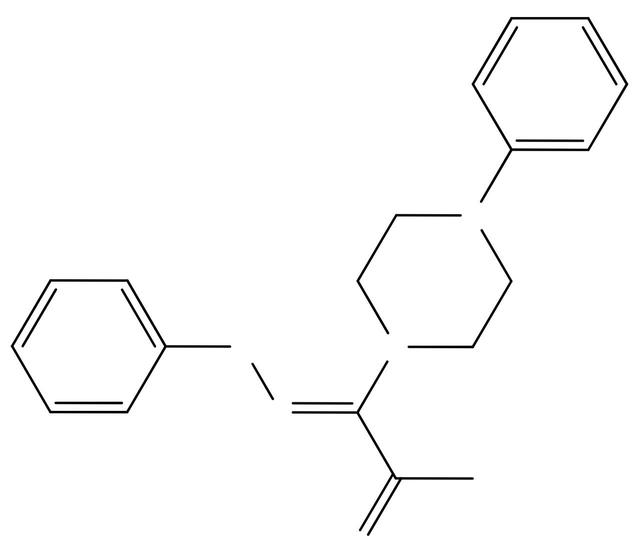
3.2.2. General procedure for the synthesis compounds (38-40)

To a stirred solution of 1-chloro-1-(4-subsitituted-phenylhydrazono)-2-propanone (**37a-d**) (10 mmol) and triethylamine (3 mL) in THF (10 mL) was added the corresponding piperazine (2 g, 25 mmol) the mixture was stirred at room temperature over night.

The reaction mixture was then diluted with water (60 mL) and extracted with diethylether (3 x 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness under reduced pressure to yield the crude title compounds which were purified by recrystalization from ethanol.

The following compounds were prepared according to the above procedure:

1-(4-Phenyl-1-piperazinyl)-1-(phenylhydrazono)-2-propanone ***(38a)***

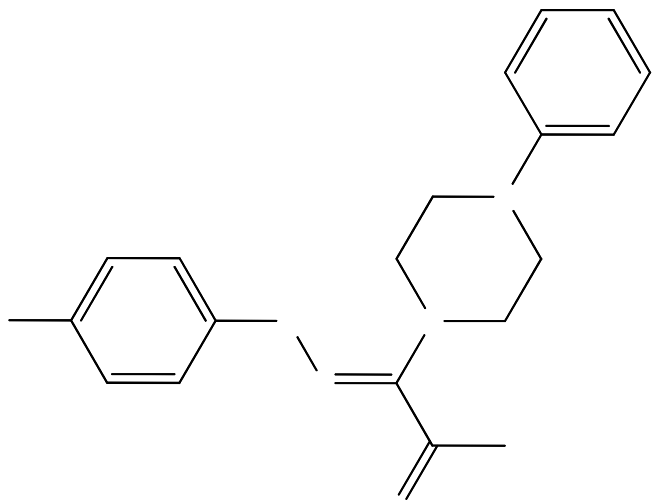


Yield = 74%; M.p = 116-118 ^{o}C (Lit. M.p 118-119 ^{o}C) (Abdel-Jalil *et al.*, 2010). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.46 (s, 3H, C*H*$_3$), 3.25 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.26 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 6.90-7.37 (m, 10H, H-2/ H-3/ H-4/ H-5/H-6/ H-2''/ H-3''/ H-4''/ H-5''/ H-6''), 9.22 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.8 (*C*H$_3$), 48.12 (C-2'/C-6'), 50.22 (C-3'/C-5'), 114.2, 116.4, 120.1, 122.3, 129.2, 129.6, 142.5, 143.2, 151.5 (Ar), 195.1 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{19}H_{23}N_4O$ $[M+H]^+$ 323.17936; found 323.18051.

3 2

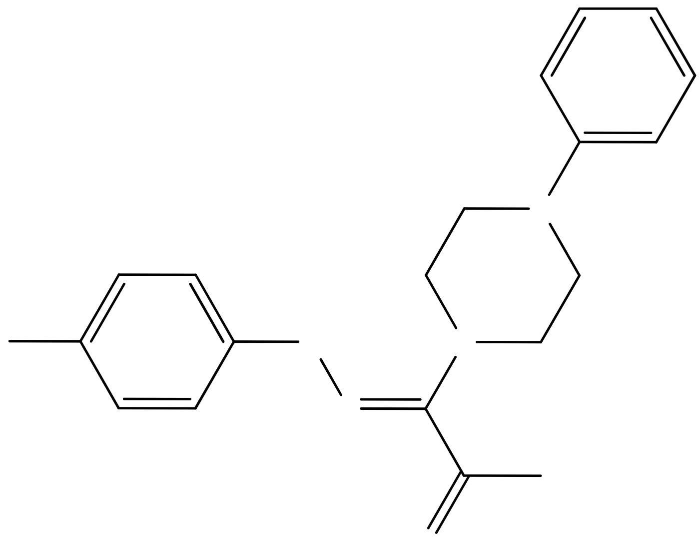
4 1

1-(4-Phenyl-1-piperazinyl)-1-(4-flourophenylhydrazono)-2-propanone ***(38b)***



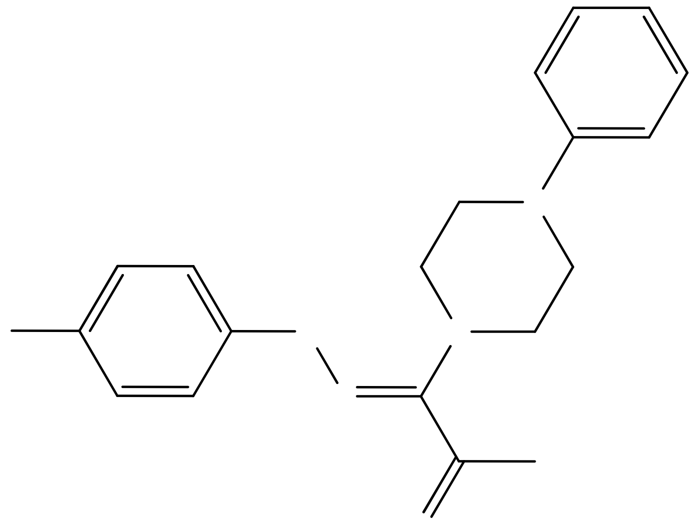
Yield = 76%; M.p = 123-125 °C. [1]H-NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, C*H*$_3$), 3.24 (bs, 4H, 2 × H-3'/ 2 ×H-5'), 3.26 (bs, 4H, 2 × H-2'/ 2 × H-6'), 6.87-7.32(m, 9H, H-2/ H-3/ H-5/ H-6/ H-2″/ H-3″/ H-4″/ H-5″/ H-6″), 9.15 (s, 1H, N-*H*). [13]C-NMR (75 MHz, $CDCl_3$): δ 25.8 (*C*H$_3$), 48.1 (C-2'/C-6'), 50.3 (C-3'/C-5'), 115.3, 115.4, 116.0, 116.3, 116.4, 120.3, 129.3, 139.0, 143.3, 151.5, 156.9, 160.1 (Ar), 195.0 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{19}H_{22}FN_4O$ $[M+H]^+$ 341.16994; found 341.16297.

1-(4-Phenyl-1-piperazinyl)-1-(4-chlorophenylhydrazono)-2-propanone ***(38c)***



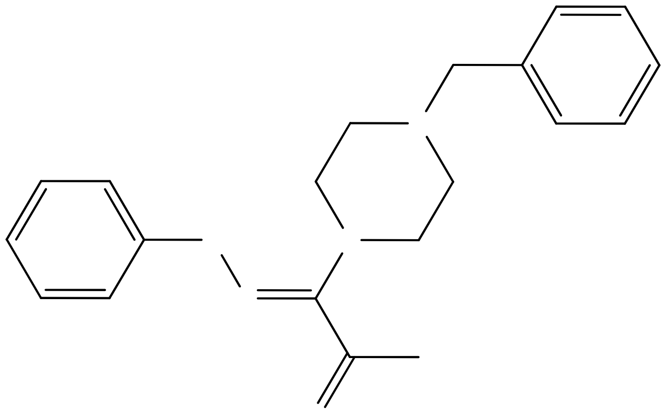
Yield = 65%; M.p = 144-146 °C (Lit. M.p 145-150 °C) (Abdel-Jalil *et al*., 2010). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, C*H*$_3$), 3.25 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.27 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 6.92–7.32 (m, 5H, H-2''/ H-6''+ H-3''/ H-5''+ H-4''), 7.13(d, *J* = 8.8 Hz, 2H, H-2/ H-6), 7.27 (d, *J* = 8.8 Hz, 2H, H-3/ H-5), 9.16 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.9 (*C*H$_3$), 47.9 (C-2'/C-6'), 50.4 (C-3'/C-5'), 114.8, 115.4, 116.7, 116.9, 117.4, 120.7, 126.9, 127.6, 129.1, 129.3, 129.5, 141.2, 141.4, 143.5 (Ar), 195.0 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{19}H_{22}ClN_4O$ $[M+H]^+$ 357.14039; found 357.13970.

1-(4-Phenyl-1-piperazinyl)-1-(4-bromophenylhydrazono)-2-propanone ***(38d)***



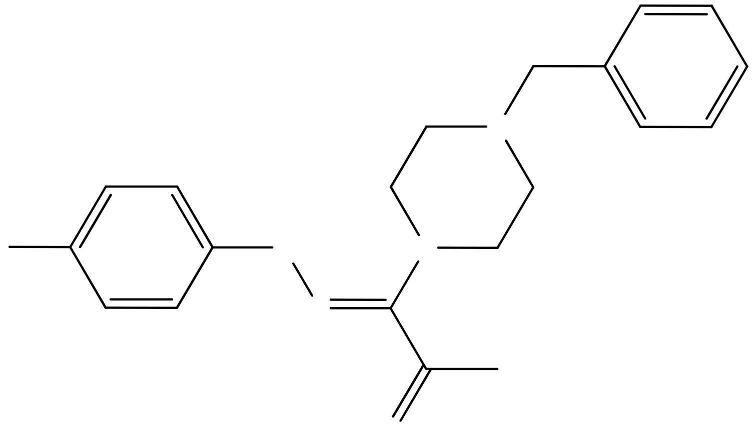
Yield = 78%; M.p = 146-148 ^{o}C (Lit. M.p 148–150 ^{o}C) (Abdel-Jalil *et al*., 2010). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 3H, C*H*$_3$), 3.28 (bs, 8H, 2 × H-2'/ 2 × H-6'/ 2 × H-3'/ 2 ×H-5'), 6.93–7.32 (m, 5H, H-2"/ H-6"+ H-3"/ H-5"+ H-4"), 7.07(d, *J* = 8.7 Hz, 2H, H-2/ H-6), 7.40 (d, *J* = 8.7 Hz, 2H, H-3/ H-5), 9.12 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.8 (*C*H$_3$), 47.8 (C-2'/C-6'), 50.5 (C-3'/C-5'), 114.4, 115.8, 116.8, 117.0, 129.3, 132.4, 141.7, 143.5, 151.4 (Ar), 195.1 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{19}H_{23}BrN_4O$ $[M+H]^+$ 401.08987; found 401.08779.

1-(4-Benzyl-1-piperazinyl)-1-(phenylhydrazono)-2-propanone ***(39a)***



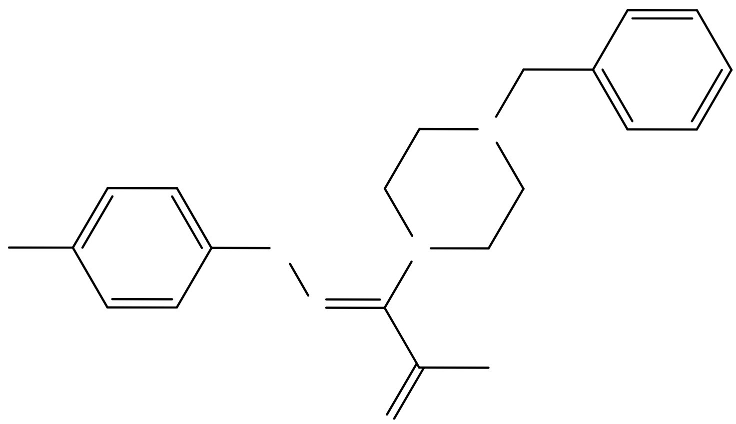
Yield = 76%; M.p = 102-105 °C. [1]H-NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H, CH_3), 2.54 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.06 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 3.57 (s, 2H, CH_2), 6.97 (t, J = 7.3, 1H, H-4), 7.17-7.37 (m, 9H, H-2/ H-3/ H-5/ H-6/ H-2/ H-3''/ H-4''/ H-5''/ H-6''), 9.13 (s, 1H, N-*H*). [13]C-NMR (75 MHz, $CDCl_3$): δ 25.7 (*C*H_3), 47.8 (C-2'/C-6'), 53.8 (C-3'/C-5'), 63.2 (*C*H_2), 114.1, 122.0, 127.1, 128.3, 129.1, 129.4, 138.0, 142.5, 143.5 (Ar), 195.0 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{20}H_{25}N_4O$ $[M+H]^+$ 337.19501; found 337.20229.

1-(4-Benzyl-1-piperazinyl)-1-(4-flourophenylhydrazono)-2-propanone ***(39b)***



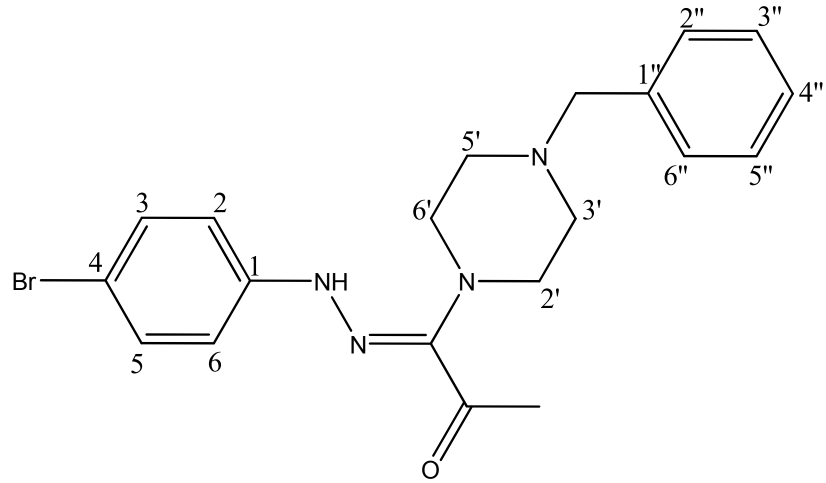
Yield = 76%; M.p = 121-124 °C. [1]H-NMR (300 MHz, $CDCl_3$): δ 2.39 (s, 3H, C*H*$_3$), 2.53 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.06 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 3.57 (s, 2H, C*H*$_2$), 6.99-7.04 (m, 2H, H-2, H-6), 7.11-7.14 (m, 2H, H-3, H-5) 7.25-7.37 (m, 5H, H-2''/ H-6''+ H-3''/ H-5''+ H-4''), 9.08 (s, 1H, N-*H*). [13]C-NMR (75 MHz, $CDCl_3$): δ 25.7 (*C*H_3), 47.8 (C-2'/C-6'), 53.8 (C-3'/C-5'), 63.2 (*C*H_2), 115.0, 115.2, 115.9, 116.2, 127.1, 128.3, 129.1, 138.0, 138.9, 143.5, 156.7, 159.8 (Ar), 194.8 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{20}H_{24}FN_4O$ $[M+H]^+$ 355.18559; found 355.19287.

1-(4-Benzyl-1-piperazinyl)-1-(4-chlorophenylhydrazono)-2-propanone ***(39c)***



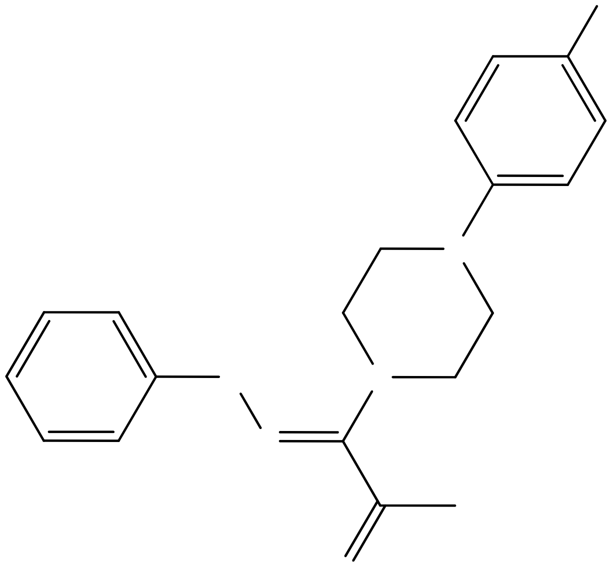
Yield = 63%; M.p = 115-117 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H, CH_3), 2.54 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.06 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 3.57 (s, 2H, CH_2), 6.92-6.95 (m, 1H, H-2), 7.01-7.04 (m, 1H, H-6), 7.20-7.37 (m, 7H, H-3/ H-5/ H-2''/ H-3''/ H-4''/ H-5''/ H-6''), 9.08 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.8 (CH_3), 47.8 (C-2'/C-6'), 53.8 (C-3'/C-5'), 63.1 (CH_2), 114.2, 127.1, 128.3, 129.0, 130.4, 135.2, 138.0, 143.8, 144.2 (Ar), 195.0 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{20}H_{24}ClN_4O$ $[M+H]^+$ 371.15604; found 371.16332.

1-(4-Benzyl-1-piperazinyl)-1-(4-bromophenylhydrazono)-2-propanone ***(39d)***



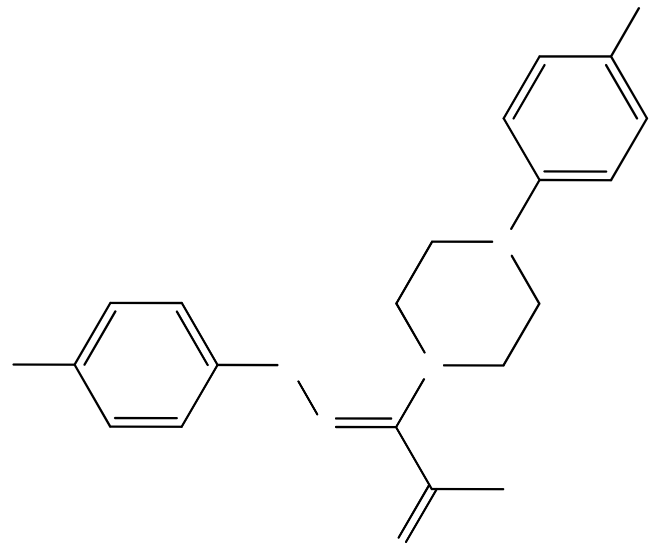
Yield = 96%; M.p = 106-108 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.39 (s, 3H, C*H*$_3$), 2.53 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.04 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 3.56 (s, 2H, C*H*$_2$), 7.06 (d, *J* = 8.7, 2H, H-2/H-6), 7.40 (d, *J* = 8.7, 2H, H-3/H-5), 7.25-7.34 (m, 5H, H-2"/ H-6"+ H-3"/ H-5"+ H-4"), 9.08 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.7 (*C*H$_3$), 47.8 (C-2'/C-6'), 53.7 (C-3'/C-5'), 63.1 (*C*H$_2$), 115.6, 127.1, 128.2, 129.1, 132.2, 138.0, 141.3, 141.7, 143.9 (Ar), 194.9 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{20}H_{24}BrN_4O$ $[M+H]^+$ 415.10552; found 415.11280**.**

1-(4-(4-Fluorophenyl)-1-piperazinyl)-1-(phenylhydrazono)-2-propanone ***(40a)***



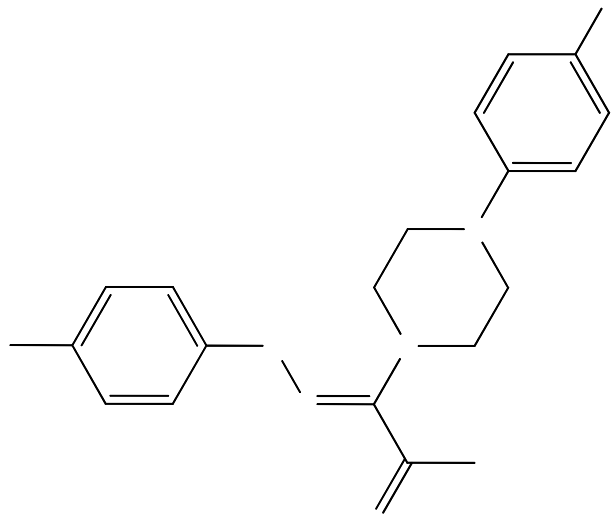
Yield = 75%; M.p = 160-162 °C (Lit. M.p 160–164 °C) (Abdel-Jalil *et al*., 2010). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, C*H*$_3$), 3.19 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.20 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 6. 89-7. 50 (m, 9H, H-2/ H-6/ H-3/ H-5/ H-2''/ H-3''/ H-4''/ H-5''/ H-6''), 9.19 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.8 (*C*H$_3$), 48.1 (C-2'/C-6'), 51.2 (C-3'/C-5'), 114.2, 115.5, 115.8, 118.1, 118.2, 122.3,129.5, 142.5, 143.1, 148.1, 148.2, 159.0 (Ar), 195.1 (C=O). HRMS(ESI) m/z: Calcd for $C_{19}H_{22}FN_4O$ $[M+H]^+$ 341.16994; found 341.1672.

1-(4-(4-Fluorophenyl)-1-piperazinyl)-1-(4-flourophenylhydrazono)-2-propanone ***(40b)***



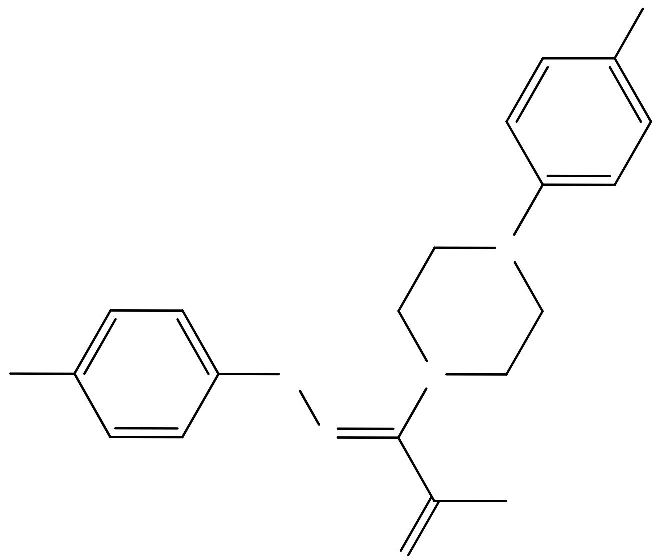
Yield = 72%; M.p = 157-159 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 3H, C*H*$_3$), 3.20 (bs, 8H, 2 × H-2'/ 2 × H-6'/ 2 × H-3'/ 2 ×H-5'), 6.88-7.17 (m, 8H, H-2/ H-3/ H-5/ H-6/ H-2''/ H-3''/ H-5''/ H-6''), 9.13 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.7 (CH_3), 48.0 (C-2'/C-6'), 51.1 (C-3'/C-5'), 115.1, 115.2, 115.4, 115.9, 116.3,118.1, 118.2, 138.8, 141.3, 143.1, 148.0, 155.8, 156.8, 158.9, 160.0 (Ar), 194.90 (C=O). HRMS (ESI) *m/z*: Calcd for $C_{19}H_{21}F_2N_4O$ $[M+H]^+$ 359.16052; found 359.15970.

1-(4-(4-Fluorophenyl)-1-piperazinyl)-1-(4-chlorophenylhydrazono)-2-propanone ***(40c)***



Yield = 83%; M.p = 148-150 ^{o}C (Lit. M.p 150–154 ^{o}C) (Abdel-Jalil *et al*., 2010). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H, C*H*$_3$), 3.18 (bs, 8H, 2 × H-2'/ 2 × H-6'/ 2 × H-3'/ 2 ×H-5'), 6.87–6.99 (m, 4H, H-2''/ H-3''/ H-5''/ H-6''), 7.11(d, *J* = 8.8 Hz, 2H, H-2/ H-6), 7.26 (d, *J* = 8.8 Hz, 2H, H-3/ H-5), 9.13 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.8 (*C*H$_3$), 48.0 (C-2'/C-6'), 51.2 (C-3'/C-5'), 114.4, 115.5, 115.8, 118.2, 118.3, 126.9, 129.5, 141.2, 143.5, 148.0, 155.8, 159.0 (Ar), 195.0 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{19}H_{21}ClFN_4O$ $[M+H]^+$ 375.13097; found 375.12872.

1-(4-(4-Fluorophenyl)-1-piperazinyl)-1-(4-bromophenylhydrazono)-2-propanone ***(40d)***



Yield = 79%; M.p = 151-153 ^{o}C (Lit. M.p 152–154 ^{o}C) (Abdel-Jalil *et al*., 2010). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H, CH_3, 3.18 (bs, 8H, 2 × H-2'/ 2 × H-6'/ 2 × H-3'/ 2 ×H-5'), 6.87–6.99 (m, 4H, H-2''/ H-3''/ H-5''/ H-6''), 7.06 (d, *J* = 8.5 Hz, 2H, H-2/ H-6), 7.40 (d, *J* = 8.5 Hz, 2H, H-3/ H-5), 9.12 (s, 1H, N-*H*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 25.8 (CH_3), 48.1 (C-2'/C-6'), 51.2 (C-3'/C-5'), 114.3, 115.5, 115.8, 115.8, 118.2, 118.3, 132.4, 141.6, 143.6, 148.0, 148.1, 155.8, 159.0 (Ar), 195.0 (C=O). HRMS(ESI) *m/z*: Calcd for $C_{19}H_{21}BrFN4O$ $[M+H]^+$ 419.08045; found 419.0772.

3.2.3. Synthesis of 6-substituted-4-methyl-3-(4-aryl-piperazin-1-yl)cinnolines (41-43 / Scheme 15)

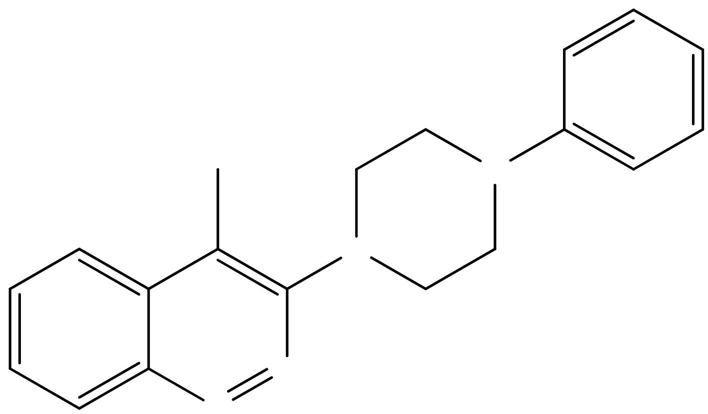
General procedure:

A solution of the corresponding amidrazones **(38-40)** (1.5 mmol) in polyphosphoric acid (PPA) (5.0 g) was stirred at 110-120 ^{o}C over night.

The reaction mixture was then cooled to room temperature, followed by the addition of 10 g of ice and neutralization with aqueous ammonium hydroxide (10 %). The mixture was then extracted with ethylacetate (3 x 50 ml) and the combined organic layers were evaporated under reduced pressure and purified by chromatography to give the title compounds.

The following were prepared according to the above general procedure:

4-Methyl-3-(4-phenylpiperazin-1-yl)cinnoline ***(41a)***

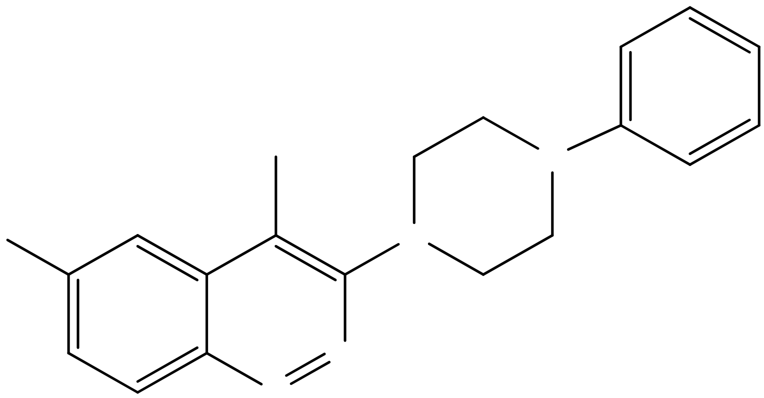


Yield = 75%; M.p = 164-166 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.64 (s, 3H, C*H*$_3$), 3.41-3.43 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.54-3.57 (m, 4H, 2 × H-2'/ 2 ×H-6'), 6.89 (t, *J* = 7.2 Hz, 1H, H-4''), 7.02 (d, 2H, *J* = 8.0 Hz, H-2''/ H-6''), 7.30 (t, J = 8.0, 2H, H-3''/H-5''), 7.62-7.65 (m, 2H, H-7/ H-5), 7.89-7.92 (m, 1H, H-6), 8.36-8.39 (m, 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.6 (*C*H$_3$), 49.5 (C-2'/C-6'), 50.9 (C-3'/C-5'), 116.2, 119.1, 122.2, 122.8, 127.9, 128.2, 129.1, 130.1, 130.3, 148.0, 151.4, 159.2 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{19}H_{21}N_4$ $[M+H]^+$ 305.16880; found 305.17607.

5 4 4a 3 6

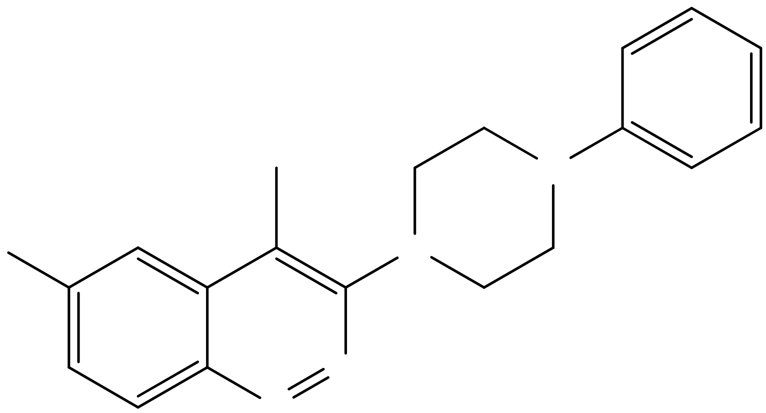
7 N 8a N 8

6-Flouro-4-methyl-3-(4-phenylpiperazin-1-yl)cinnoline ***(41b)***



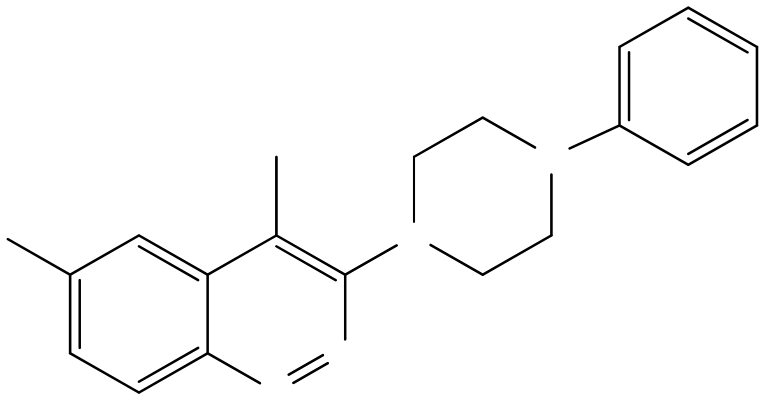
Yield = 78%; M.p = 196-198 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.58 (s, 3H, CH_3), 3.41-3.44 (m, 4H, 2 × H-3'/ 2 ×H-5'), 3.55-3.59 (m, 4H, 2 × H-2'/ 2 × H-6'), 6.89 (t, J = 7.2 Hz, 1H, H-4''), 7.02 (d, 2H, J = 8.1 Hz, H-2''/ H-6''), 7.28-7.33 (m, 2H, H-3''/H-5''), 7.37-7.47 (m, 2H, H-7/ H-5), 8.37-8.43 (m, 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.0 (CH$_3$), 49.6 (C-2'/C-6'), 50.9 (C-3'/C-5'), 105.7, 106.0, 116.3, 119.1, 119.5, 120.0, 121.2, 129.3, 129.8, 133.6, 133.8, 145.9, 151.5, 159.2, 161.2, 164.6 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{19}H_{20}FN_4$ $[M+H]^+$ 323.15937; found 323.16665.

6-Chloro-4-methyl-3-(4-phenylpiperazin-1-yl)cinnoline ***(41c)***



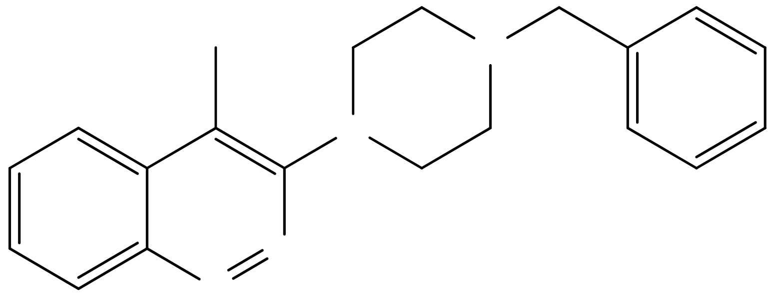
Yield = 43%; M.p = 156-160 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.95 (s, 3H, CH_3), 3.40-3.44 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.54-3.60 (m, 4H, 2 × H-2'/ 2 ×H-6'), 6.78-6.97 (m, 1H, H-4''), 7.02 (d, 2H, J = 7.9, H-2''/ H-6''), 7.26-7.33 (m, 2H, H-3''/H-5''), 7.46-7.58 (m, 1H, H-7), 7.67 (d, J = 7.2, 1H, H-5), 8.30-8.36 (m, 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 17.7 (CH_3), 49.5 (C-2'/C-6'), 50.7 (C-3'/C-5'), 116.2, 119.9, 124.6, 127.3, 128.4, 129.1, 130.2, 131.5, 132.5, 149.1, 151.3, 160.7 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{19}H_{19}ClN_4Na$ $[M+Na]^+$ 361.11959; found 361.11905.

6-Bromo-4-methyl-3-(4-phenylpiperazin-1-yl)cinnoline ***(41d)***



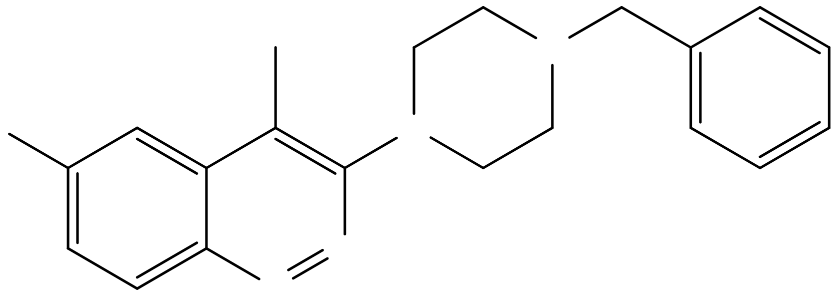
Yield = 20%; M.p = 194-196 °C. [1]H-NMR (300 MHz, $CDCl_3$): δ 2.59 (s, 3H, CH_3), 3.37-3.48 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.52 -3.61 (m, 4H, 2 × H-2'/ 2 ×H-6'), 6.89 (t, J = 7.2, 1H, H-4''), 7.02 (d, J = 8.0, 2H, H-2''/ H-6''), 7.25-7.33 (m, 2H, H-3''/ H-5''), 7.69 (d, J = 9.0, 1H, H-7), 8.10 (s, 1H, H-5), 8.24 (d, J = 9.0, 1H, H-8). [13]C-NMR (75 MHz, $CDCl_3$): δ 12.8 (CH_3), 49.5 (C-2'/C-6'), 50.9 (C-3'/C-5'), 116.2, 120.0, 120.6, 125.3, 125.6, 129.2, 131.6, 132.0, 132.7, 146.4, 151.4, 159.5 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{19}H_{19}BrN_4Na$ $[M+Na]^+$ 405.06908; found 405.06853.

3-(4-Benzylpiperazin-1-yl)-4-methylcinnoline ***(42a)***



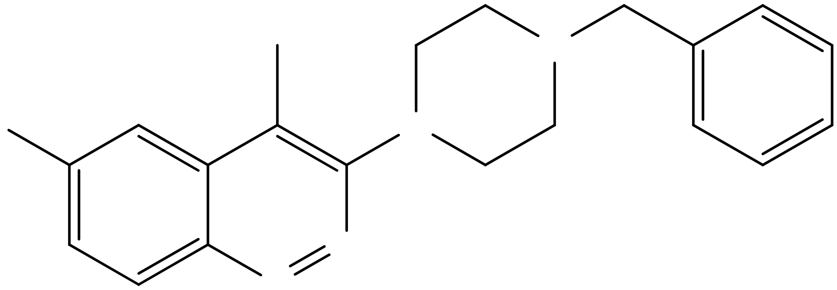
Yield = 30%; M.p = 162-164 °C. [1]H-NMR (300 MHz, $CDCl_3$): δ 2.58 (s, 3H, C*H*$_3$), 2.69-2.72 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.40-3.43 (m, 4H, 2 × H-2'/ 2 ×H-6'), 3.63 (s, 2H, C*H*$_2$), 7.24-7.40 (m, 5H, H-2''/ H-6''+ H-3''/ H-5''+ H-4''), 7.58-7.64 (m, 2H, H-5/ H-7), 7.84-7.89 (m, 1H, H-6), 8.32-8.37 (m, 1H, H-8). [13]C-NMR (75 MHz, $CDCl_3$): δ 12.8 (*C*H$_3$), 50.9 (C-2'/C-6'), 53.5 (C-3'/C-5'), 63.3 (*C*H$_2$), 121.8, 123.0, 127.2, 127.8, 128.4 (2x), 129.3, 130.3, 132.9, 138.1, 148.0, 159.5 (Ar). HRMS(ESI) *m/z*: Calcd for $C_{20}H_{23}N_4$ $[M+H]^+$ 319.18445; found 319.19172.

*3-(4-Benzylpiperazin-1-yl)-6-flouro-4-methylcinnoline****(42b)***



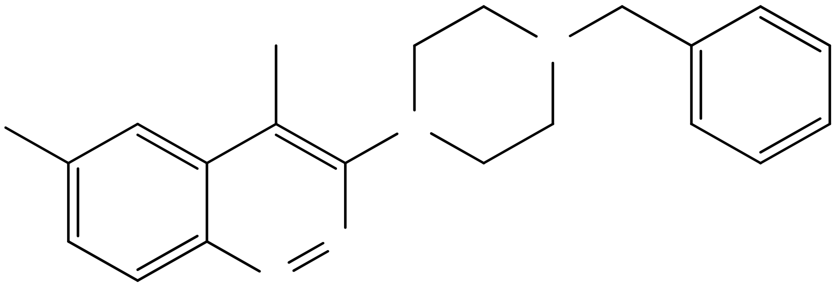
Yield = 20%; M.p = 113-117 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H, C*H*$_3$), 2.70 (bs, 4H, 2 × H-3'/ 2 × H-5'), 3.42 (bs, 4H, 2 × H-2'/ 2 ×H-6'), 3.62 (s, 2H, CH_2), 7.25-7.37 (m, 7H, H-5/ H-7/ H-2"/ H-3"/ H-4"/ H-5" /H-6"), 8.33-8.38 (m, 1H, H-8), ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.0 (*C*H$_3$), 50.6 (C-2'/C-6'), 53.3 (C-3'/C-5'), 63.2 (*C*H$_2$), 105.5, 105.8, 118.7, 119.1, 120.8, 120.9, 127.2, 128.3, 129.2, 129.8, 133.5, 133.6, 137.9, 145.7, 159.3, 161.0, 164.4 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{20}H_{22}FN_4$ $[M+H]^+$ 337.17502; found 337.18230.

3-(4-Benzylpiperazin-1-yl)-6-chloro-4-methylcinnoline ***(42c)***



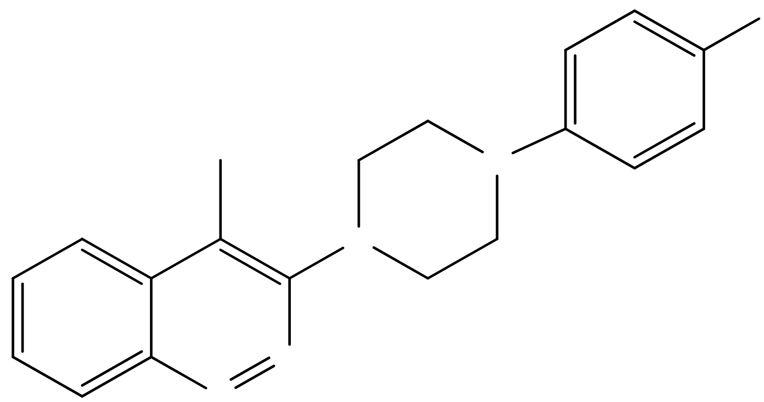
Yield = 60%; M.p = 150-152 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.53 (s, 3H, CH_3), 2.68-2.71 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.41-3.44 (m, 4H, 2 × H-2'/ 2 ×H-6'), 3.62 (s, 2H, CH_2), 7.25-7.39 (m, 5H, H-2"/ H-6"+ H-3"/ H-5"+ H-4"), 7.51 (dd, 1H, *J* = 9.0, 1.9, H-7), 7.84 (d, *J* = 1.9, 1H, H-5), 8.28 (d, *J* = 9.0, 2H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.9 (CH_3), 50.7 (C-2'/C-6'), 53.3 (C-3'/C-5'), 63.2 (CH_2), 120.3, 121.7, 127.1, 128.3, 128.9, 129.0, 129.2, 132.0, 136.6, 138.0, 146.1, 159.6 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{20}H_{22}ClN_4$ $[M+H]^+$ 353.14547; found 353.15275.

3-(4-Benzylpiperazin-1-yl)-6-bromo-4-methylcinnoline ***(42d)***



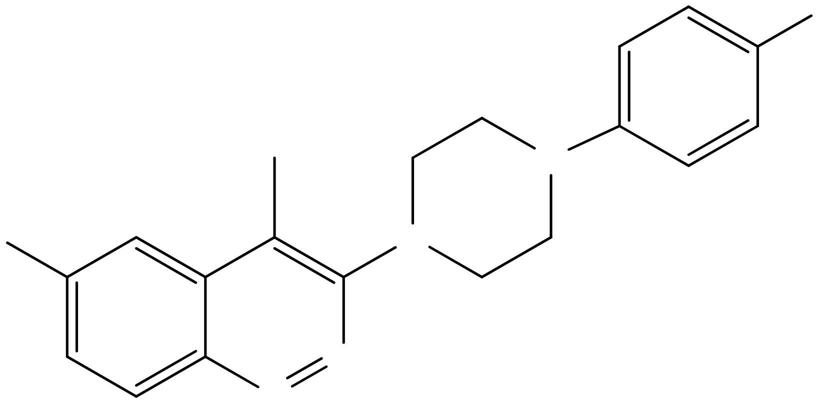
Yield = 45%; M.p = 153-155 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.52 (s, 3H, C*H*$_3$), 2.68-2.71 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.41- 3.44 (m, 4H, 2 × H-2'/ 2 ×H-6'), 3.62 (s, 2H, C*H*$_2$), 7.24-7.39 (m, 5H, H-2''/ H-6''+ H-3''/ H-5''+ H-4''), 7.64 (dd, 1H, *J* = 9.0, 1.9 , H-7), 8.03 (d, *J* = 1.9, 1H, H-5), 8.20 (d, *J* = 9.0, 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.0 (*C*H$_3$), 50.8 (C-2'/C-6'), 53.4 (C-3'/C-5'), 63.3 (*C*H$_2$), 120.2, 125.3, 125.5, 127.2, 128.4, 129.3, 129.5, 131.4, 132.0, 138.0, 146.3, 159.7 (Ar). HRMS (ESI) *m/z*: Calcd for $C_{20}H_{21}BrN_4Na$ $[M+Na]^+$ 419.08473; found 419.08418.

3-(4-(4-Fluorophenyl)piperazin-1-yl)-4-methylcinnoline ***(43a)***



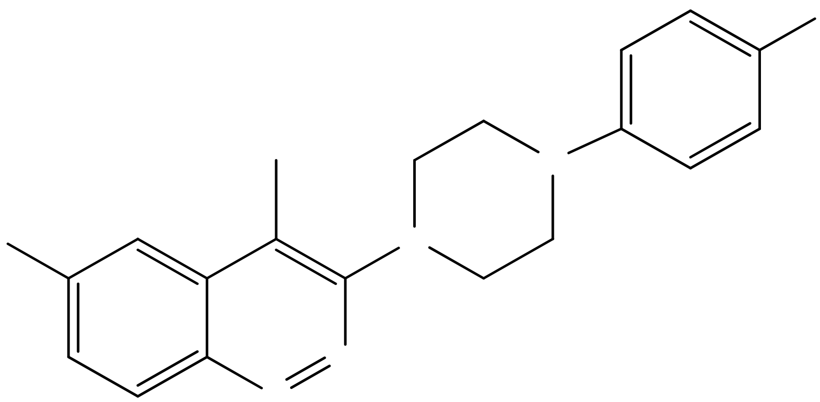
Yield = 35%; M.p = 183-185 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.64 (s, 3H, CH_3), 3.33-3.37 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.54-3.57 (m, 4H, 2 × H-2'/ 2 ×H-6'), 6.95-7.03 (m, 4H, H-2"/ H-3"/ H-5"/ H-6"), 7.63-7.66 (m, 2H, H-5/ H-7), 7.89-7.92 (m, 1H, H-6), 8.36-8.40 (m, 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.6 (CH_3), 50.5 (C-2'/C-6'), 51.0 (C-3'/C-5'), 115.4, 115.7, 117.9, 118.0, 122.1, 122.9, 127.9, 128.2, 130.2, 130.3, 148.1 (2x), 155.7, 158.8, 159.1 (Ar). (ESI) *m/z*: Calcd for $C_{19}H_{20}FN_4$ $[M+H]^+$ 323.15937; found 323.16665.

6-Flouro -3-(4-(4-fluorophenyl)piperazin-1-yl)-4-methylcinnoline ***(43b)***



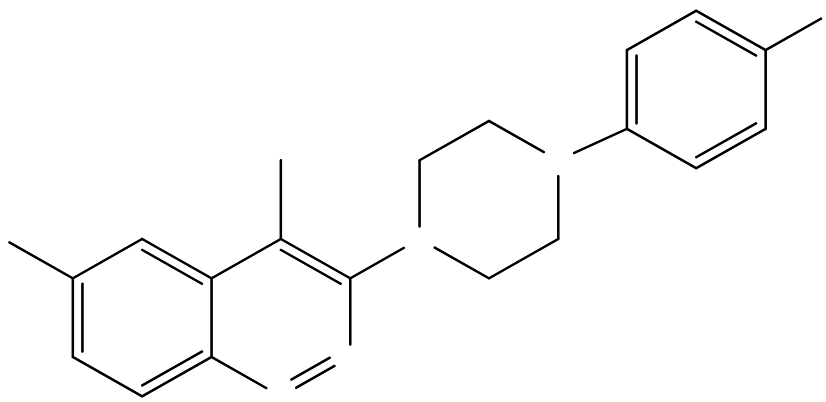
Yield = 25%; M.p = 173-176 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.57 (s, 3H, CH_3), 3.33-3.36 (m, 4H, 2 × H-3'/ 2 × H-5'), 3.55- 3.58 (m, 4H, 2 × H-2'/ 2 ×H-6'), 6.93-7.03 (m, 4H, H-2''/ H-3''/ H-5''/ H-6''), 7.37-7.46 (m, 2H, H-5/ H-7), 8.40 (dd, J = 9.2, 5.7 , 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.1 (CH_3), 50.6 (C-2'/C-6'), 50.9 (C-3'/C-5'), 105.7, 106.0, 115.5, 115.8, 118.1, 118.2, 119.2, 119.6, 121.4, 133.6, 133.7, 145.9, 148.1, 153.7, 155.8, 159.0, 159.2, 161.3, 164.6 (Ar). (ESI) *m/z*: Calcd for $C_{19}H_{19}F_2N_4$ $[M+H]^+$ 341.14995; found 341.15723.

6-Chloro-3-(4-(4-fluorophenyl)piperazin-1-yl)-4-methylcinnoline ***(43c)***



Yield = 75%; M.p = 156-158 °C. ¹H-NMR (300 MHz, $CDCl_3$): δ 2.59 (s, 3H, C*H*$_3$), 3.33-3.36 (m, 4H, 2 × H-3'/ 2 ×H-5'), 3.55- 3.58 (m, 4H, 2 × H-2'/ 2 × H-6'), 6.91-7.03 (m, 4H, H-2''/ H-3''/ H-5''/ H-6''), 7.69 (d, *J* = 9.0, 1H,H-7), 8.08 (s, 1H, H-5), 8.69 (d, *J* = 9.0, 1H, H-8). ¹³C-NMR (75 MHz, $CDCl_3$): δ 12.9 (*C*H$_3$), 50.6 (C-2'/C-6'), 50.9 (C-3'/C-5'), 115.5, 115.7, 118.0, 118.1, 120.3, 121.8, 129.0, 129.2, 131.8, 136.8, 146.1, 147.5, 155.8, 158.9, 159.8 (Ar). (ESI) *m/z*: Calcd for $C_{19}H_{19}ClFN_4$ $[M+H]^+$ 357.12040; found 357.12010.

6-Bromo -3-(4-(4-fluorophenyl)piperazin-1-yl)-4-methylcinnoline ***(43d)***



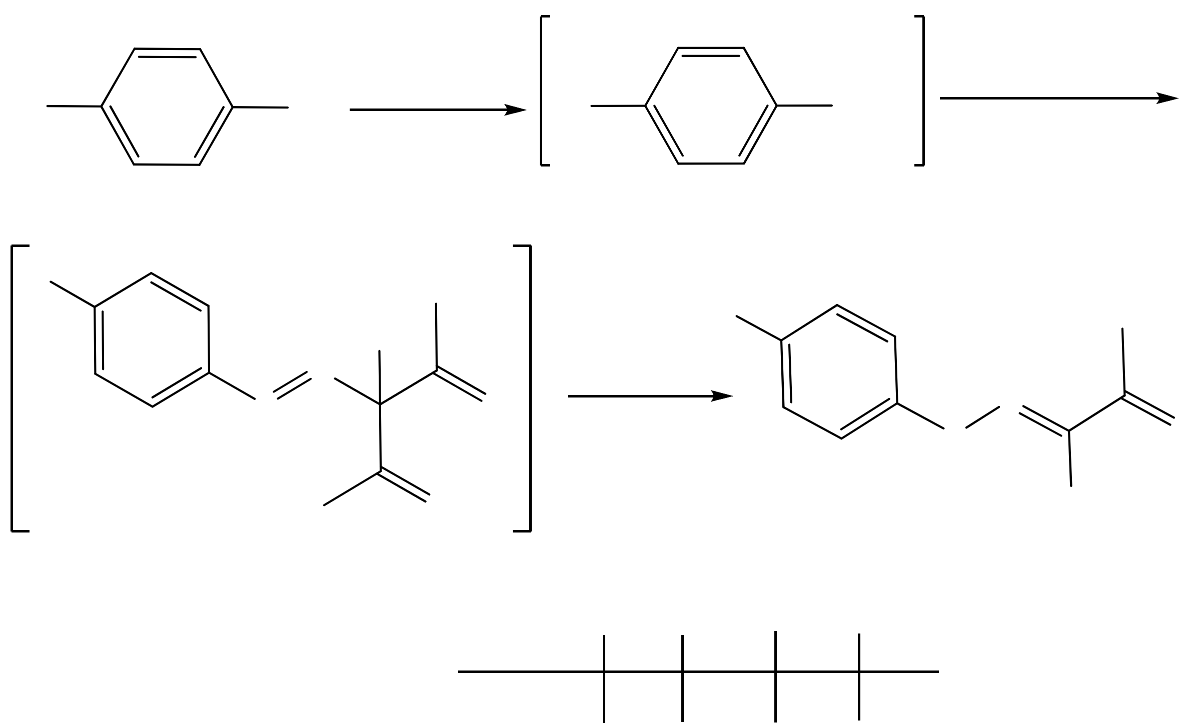
Yield = 62%; M.p = 208-210 ^{o}C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.57 (s, 3H, CH_3), 3.32-3.35 (m, 4H, 2 × H-3′/ 2 × H-5′), 3.54-3.57 (m, 4H, 2 × H-2′/ 2 ×H-6'), 6.92-7.02 (m, 4H, H-2''/ H-3''/ H-5''/ H-6''), 7,68 (dd, *J* = 9.0, 1.9, 1H, H-7), 8.06 (d, *J* = 1.9, 1H, H-5), 8.23 (d, *J* = 9.0, 1H, H-8). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.8 (CH_3), 50.5 (C-2'/C-6'), 50.8 (C-3'/C-5'), 115.4, 115.7, 117.9, 118.0, 120.5, 125.2, 125.6, 129.4, 131.6, 131.9, 146.4, 148.0, 155.7, 158.9, 159.4 (Ar). (ESI) *m/z*: Calcd for $C_{19}H_{19}BrFN_4$ $[M+H]^+$ 401.06989; found 401.07716.

4. Results and discussion

4.1. Synthesis

4.1.1. Synthesis of 1-chloro-1-(4-substituted phenylhydrazono)-2-propanone (37a-d)

The 1-chloro-1-(4-substituted phenylhydrazono)-2-propanones **(37a-d)** were prepared through diazotization of 4-substituted anilines **(36a-d)**, followed by coupling with α-chloroacetylacetone *via* Japp-Klingemann reaction. The crude product was recrystallized from acetonitrile to afford compounds **37a-d** (Scheme 11).



Scheme 11. Synthesis of compounds **37a-d** *via* Japp-Klingemann reaction.

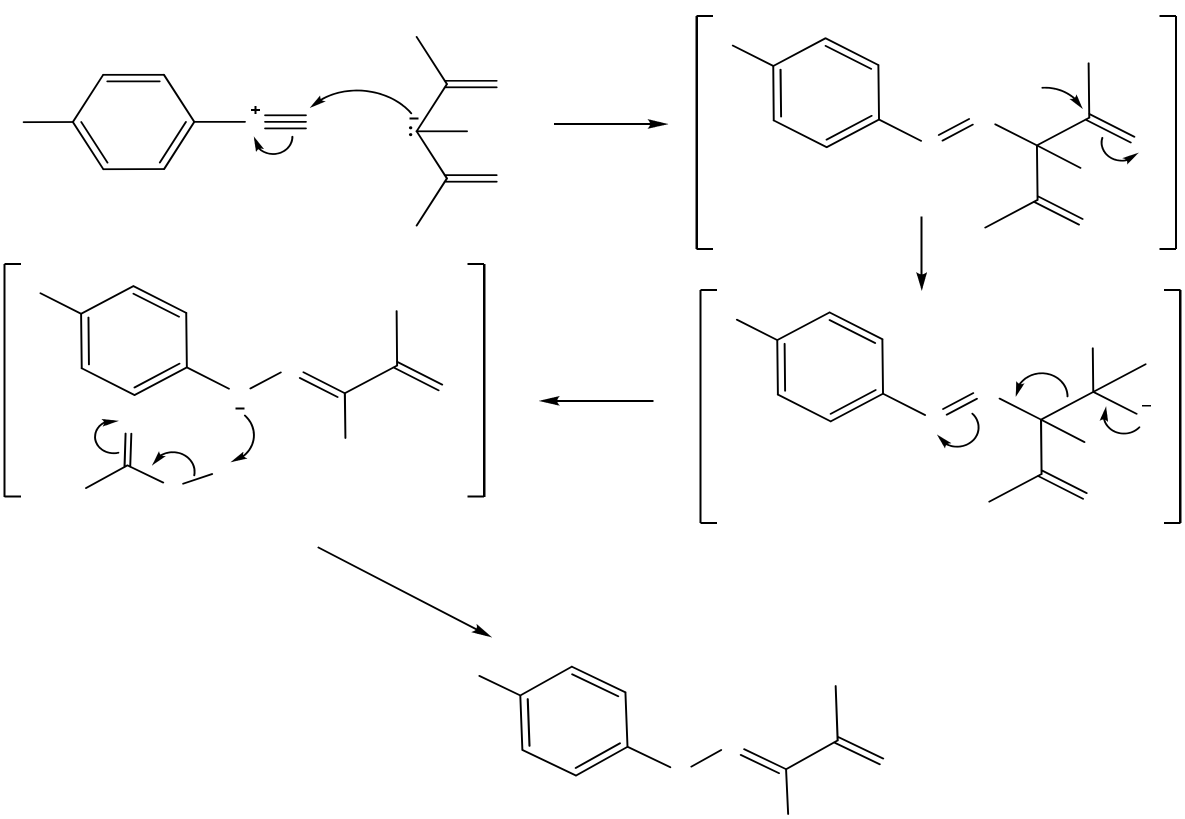
X

NH_2

$NaNO_2$

HCl, 0-5 ^oC

As shown in Scheme 12, the Japp-Klingemann coupling reaction of arene diazonium salts with activated methinyl species is performed by direct nucleophilic attack of the methine carbanion (of chloroacetylacetone) onto electrophilic nitrogen of the diazonium cation to form the respective azo compound. The latter unstable intermediate is converted to a hydrazone structure through loss of acetyl group in presence of hydroxide ion (Yao and Resnick, 1962). The melting points of the prepared compounds (**37a-d**) are in agreement with the reported melting points (El-Abadelah *et al.*, 1991).



Scheme 12. Japp-Klingemann reaction mechanism.

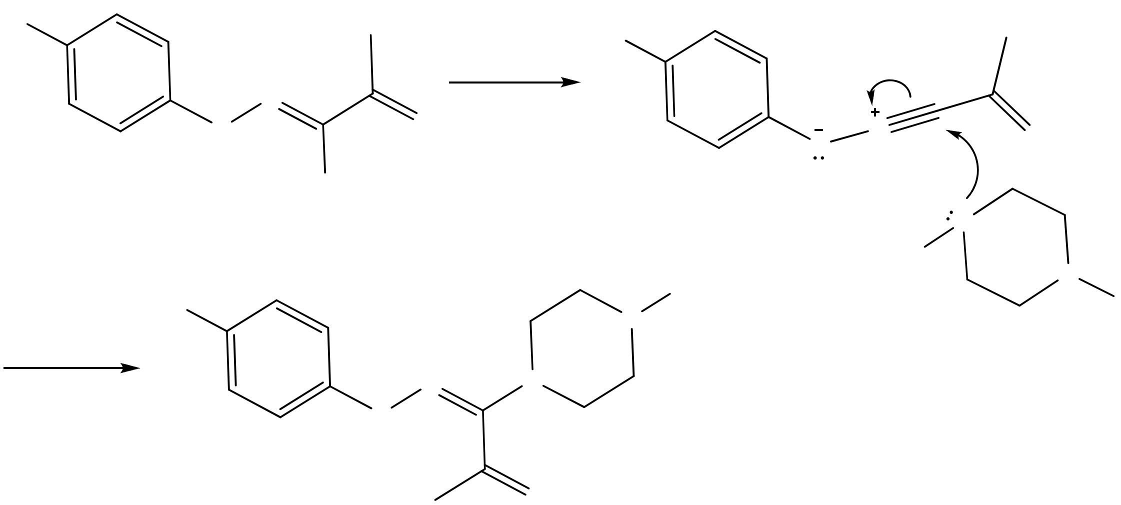
X N N Cl

O

X

4.1.2. Synthesis of 1-(2-(4-substituted-phenyl)hydrazono)-1-(4-aryl-piperazinyl)-2-propanones (38-40)

The title intermediates **38-40** were synthesized by reaction of 1-chloro-1-(4-substituted phenylhydrazono)-2-propanone **(37)** with the corresponding *N*-substituted piperazines in presence of triethylamine (TEA) (Scheme 13). The synthesis was achieved by *in situ* formation of nitrile imines from the hydrazonyl chloride derivatives, followed by nucleophilic addition of piperazine moieties (net nucleophilic substitution reaction) at room temperature to afford the title products of amidrazone derivatives.



Scheme 13. Mechanism and synthesis of amidrazone derivatives.

X

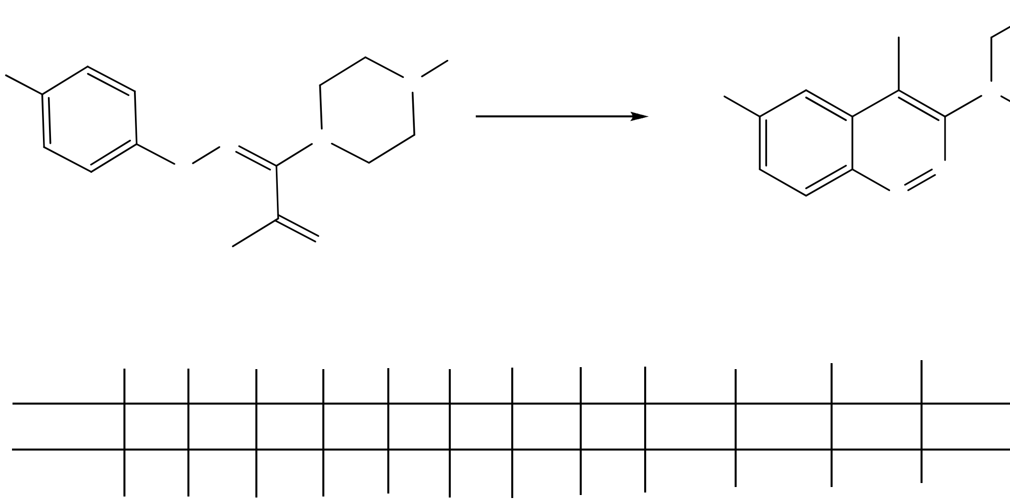
TEA

N

THF or Et

4.1.3. Synthesis of 6-substituted-4-methyl-3-(4-aryl piperazin-1-yl) cinnolines (41-43)

The synthesis of 6-substituted-4-methyl-3-(4-aryl-piperazin-1-yl) cinnolines **(41-43)** were carried out *via* interamolecular cycliazation of the respective amidrazones **(38-40)** using polyposphoric acid (PPA) as a cyclizing agent. The present method offers an easy access to 3-substituted cinnolines **(41-43** / Scheme 14**)** of potential biological applications.

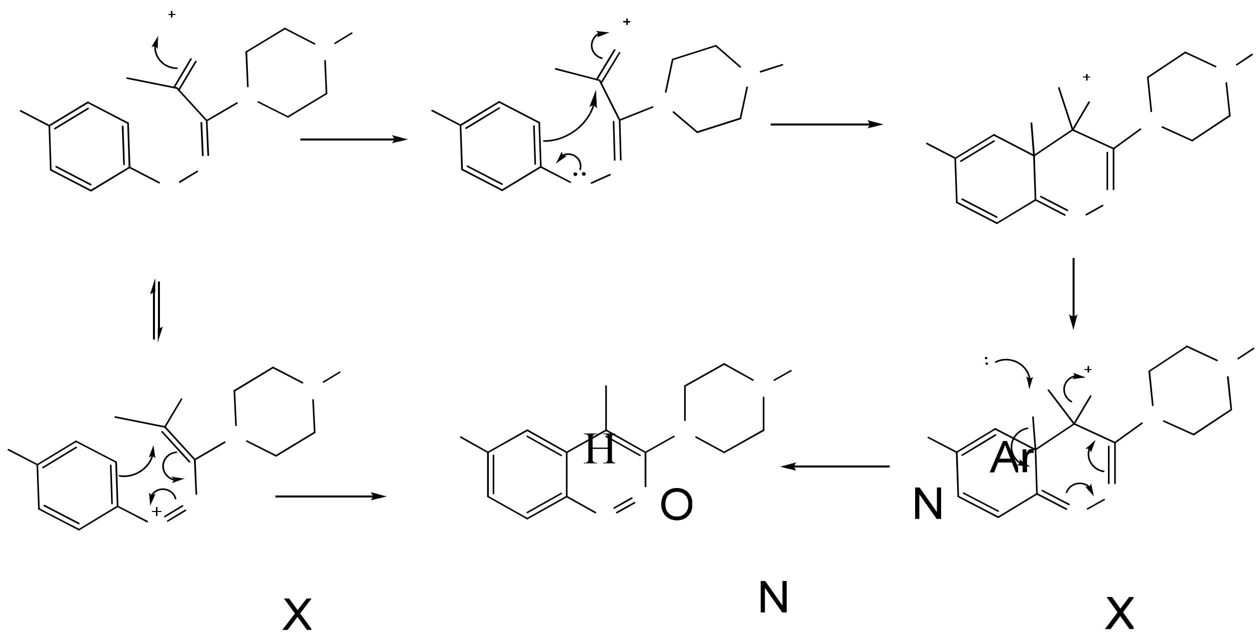


Scheme 14. Synthesis of **41-43**.

Two mechanisms were described cinnolines for the cyclization of compounds **38-40**. The first approach was nucleophilic attack by the aromatic ring π-electron of the protonated amidrazone moiety on the acyl carbonyl carbon under acidic condition, followed by elimination of water to yield the corresponding cinnoline (Scheme 15). The second mechanism involves an initial isomerization which then undergoes 6π electrocyclization *via*

38-40

aromatic six membered transition state followed by dehydration to give the cinnoline (Scheme 15) (Al-Awadi *et.al*, 2001).



Scheme 15. The mechanisms of synthesis of cinnoline derivatives.

4.2. Nuclear magnetic resonance (^{1}H & ^{13}C-NMR) spectral data of compounds 40-43

38-40

The ^{1}H & ^{13}C-NMR spectra of the new compounds are in agreement with the proposed structures as illustrated below.

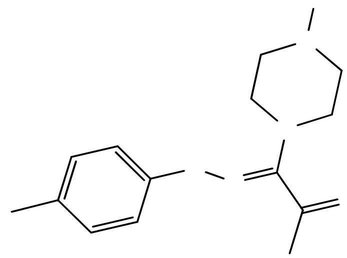
4.2.1. 1-(2-(4-Substituted phenyl)hydrazono)-1-(4-aryl-piperazinyl)-2-propanones (38-40)

The ^{1}H-NMR ($CDCl_3$) spectra of the title compounds show a singlet peak around δ 2.39-2.43 ppm corresponding to the methyl protons, CO-CH_3. The methylene protons of the piperazine moiety appear as two broad singlets between δ 2.53-3.24 and δ 3.04-3.26

assigned to (H-2'/ H- 6') and (H-3'/H-5'), respectively, while the N-*H* proton resonates as singlet between δ 9.08-9.15 ppm (Abdel-Jalil *et al*., 2010). The resonance peaks between δ 6.87 and δ 7.37 indicate the presence of aromatic protons.

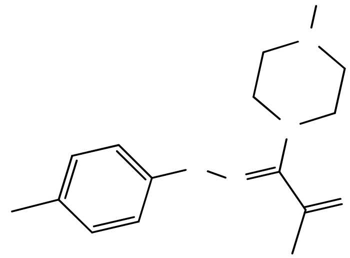
In the ^{13}C-NMR ($CDCl_3$) spectra, the methyl carbon (CO-CH_3) resonates around δ 25.7-25.8 ppm, and the methylene carbons of the piperazine moiety appear between δ 47.8-48.1 and 50.3-53.8 ppm assigned to (C2'/C6') and (C3'/C5'), respectively. The aromatic carbon signals appear between 114.1 and 160.1 ppm, while the C=O carbon signal appears around δ 194.8-195.0 ppm. The chemical shifts of the protons and carbons are illustrated in Tables 2 and 3, respectively.

Table 1. The chemical shift values (δ) and coupling constants of the different protons of the new compounds (**38b, 39a-d and 40b**)



No.	Ar	X	**COC*H*$_3$** (s, 3H)	**H-3′/H-5′** (bs, 4H)	**H-2′/H-6′** (bs, 4H)	Other peaks	**N-*H* (s, 1H)**
38b	C_6H_5-	F	2.43	3.24	3.26	6.87-7.32 (m, 9H, H-2/ H-3/ H-5/ H-6/ H-2″/ H-3″/ H-4″/ H-5″/ H-6″)	9.15
39a	$C_6H_5CH_2$-	H	2.41	2.54	3.06	3.57 (s, 2H, C*H*$_2$), 6.97 (t, *J* = 7.3, 1H, H-4), 7.17-7.37 (m, 9H, H-2/ H-3/ H-5/ H-6/ H-2″/ H-3″/ H-4″/ H-5″/ H-6″)	9.14
39b	$C_6H_5CH_2$	F	2.39	2.53	3.06	3.57 (s, 2H, C*H*$_2$), 6.99-7.04 (m, 2H, H-2, H-6), 7.11-7.14 (m, 2H, H-3, H-5), 7.25-7.37 (m, 5H, H-2″/ H-3″/ H-4″/ H-5″/ H-6″)	9.08
39c	$C_6H_5CH_2$	Cl	2.41	2.54	3.06	3.57 (s, 2H, C*H*$_2$), 6.92-6.95 (m, 1H, H-2), 7.01-7.04 (m, 1H, H-6), 7.20-7.37 (m, 7 H, H-3/ H-5/ H-2″/ H-3″/ H-4″/ H-5″/ H-6″)	9.08
39d	$C_6H_5CH_2$-	Br	2.39	2.53	3.04	3.56 (s, 2H, C*H*$_2$), 7.06 (d, *J* = 8.7, 2H, H-2/H-6), 7.40 (d, *J* = 8.7, 2H, H-3/H-5) 7.25-7.34 (m, 5H, H-2″/ H-3″/ H-4″/ H-5″/ H-6″)	9.08
40b	*4*- C_6H_4 F-	F	2.42	3.20		6.88-7.17 (m, 8H, H-2/ H-3/ H-5/ H-6/ H-2″/ H-3″/ H-5″/ H-6″)	9.13

Table 2. The chemical shift values (δ) of the different carbons of the new compounds (**38b, 39a-d and 40b**)



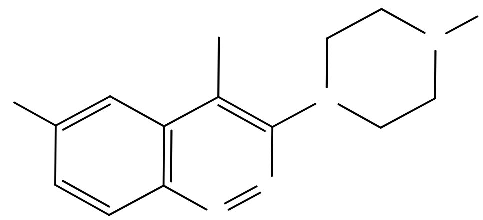
No.	**Ar**	**X**	**$COCH_3$**	**C_2'/C_6'**	**C_3'/C_5'**	**Other peaks**	**C=O**
38b	C_6H_5-	F	25.8	48.1	50.3	115.3, 115.4, 116.0, 116.3, 116.4, 120.3, 129.3, 139.0, 143.3, 151.5, 156.9, 160.1	195.0
39a	$C_6H_5CH_2$-	H	25.7	47.8	53.8	63.2 (CH2), 114.1, 122.0, 127.1, 128.3, 129.1, 129.4, 138.0, 142.5, 143.5	195.0
39b	$C_6H_5CH_2$-	F	25.7	47.8	53.8	63.2 (CH_2), 115.0, 115.2, 115.9, 116.2, 127.1, 128.3, 129.1, 138.0, 138.9, 143.5, 156.7, 159.8	194.8
39c	$C_6H_5CH_2$-	Cl	25.8	47.8	53.8	63.1 (CH_2), 114.2, 127.1, 128.3, 129.0, 130.4, 135.2, 138.0, 143.8, 144.2	195.0
39d	$C_6H_5CH_2$-	Br	25.7	47.8	53.7	63.1 (CH_2), 115.6, 127.1, 128.2, 129.1, 132.2, 138.0, 141.3, 141.7, 143.9	194.9
40b	*p*-C_6H_4F-	F	25.7	48.0	51.1	115.1, 115.2, 115.4, 115.9, 116.3, 118.1, 118.2, 138.8, 141.3, 143.1, 148.0, 155.8, 156.8, 158.9, 160.0	194.9

4.2.2. 6-Substituted-4-methyl-3-(4-aryl-piperazin-1-yl)cinnolines (41-43)

In the ^{1}H-NMR ($CDCl_3$) spectra of compounds **41-43**, a singlet peak appears between δ 2.50-2.95 ppm corresponding to the methyl protons. The methylene protons of the piperazine moiety appear as two broad singlets or multiplet peakes in the range δ 3.32-3.48 ppm and δ 2.68-3.61 ppm assigned to (H-2'/ H-6') and (H-3'/ H-5'), respectively. The aromatic protons signals appear around δ 6.78-8.69 ppm.

In the ^{13}C-NMR spectra of compounds **41-43**, the methyl (CH_3) carbon displayed upfield between δ 12.6-17.7 ppm is indication of the formation of cyclized product through acylation of the benzene ring, and the methylene carbons of the piperazine moiety appear around δ 49.5-50.9 and 50.7-53.5 ppm assigned to (C-2'/C-6') and (C-3'/C-5'), respectively. The aromatic carbons resonate around δ 105.5-164.6. The ^{1}H-NMR and ^{13}C-NMR spectra of the title compounds are in agreement with the assigned structures, and the chemical shifts are given in Tables 4 and 5, respectively.

Table 4. The chemical shift values (δ) and coupling constants of the different protons of compounds (**41-43**)

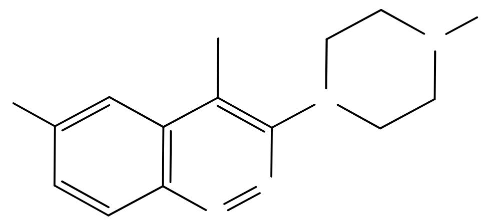


No.	Ar	X	-CH_3 (s, 3H)	H-3'/H-5' (m, 4H)	H-2'/H-6' (m, 4H)	Other peaks
41a	C_6H_5-	H	2.64	3.41-3.43	3.54-3.57	6.89 (t, *J* = 7.2 Hz, 1H, H-4"), 7.02 (d, 2H, *J* = 8.0 Hz, H-2"/ H-6"), 7.30 (t, *J* = 8.0, 2H, H-3"/H-5"), 7.62-7.65 (m, 2H, H-7/ H-5), 7.89-7.92 (m, 1H, H-6), 8.36-8.39 (m, 1H, H-8)
41b	C_6H_5-	F	2.58	3.41-3.44	3.55-3.59	6.89 (t, *J* = 7.2 Hz, 1H, H-4"), 7.02 (d, 2H, *J* = 8.1 Hz, H-2"/ H-6"), 7.28-7.33 (m, 2H, H-3"/H-5"), 7.37-7.47 (m, 2H, H-7/ H-5), 8.37-8.43 (m, 1H, H-8)
41c	C_6H_5-	Cl	2.95	3.40-3.44	3.54-3.60	6.78-6.97 (m, 1H, H-4"), 7.02 (d, 2H, *J* = 7.9, H-2"/ H-6"), 7.26-7.33 (m, 2H, H-3"/H-5"), 7.46-7.58 (m, 1H, H-7), 7.67 (d, *J* = 7.2, 1H, H-5), 8.30-8.36 (m, 1H, H-8)
41d	C_6H_5-	Br	2.59	3.37-3.48	3.52 -3.61	6.89 (t, *J* = 7.2, 1H, H-4"), 7.02 (d, *J* = 8.0, 2H, H-2"/ H-6"), 7.25-7.33 (m, 2H, H-3"/ H-5"), 7.69 (d, *J* = 9.0, 1H, H-7), 8.10 (s, 1H, H-5), 8.24 (d, *J* = 9.0, 1H, H-8)
42a	$C_6H_5CH_2$-	H	2.58	2.69-2.72	3.40-3.43	3.63 (s, 2H, CH_2), 7.24-7.40 (m, 5H, H-2"/ H-3"/ H-4"/ H-5"/ H-6"), 7.58-7.64 (m, 2H, H-5/ H-7), 7.84-7.89 (m, 1H, H-6), 8.32-8.37 (m, 1H, H-8)
42b	$C_6H_5CH_2$-	F	2.50	2.70 (bs)	3.42 (bs)	3.62 (s, 2H, CH_2), 7.25-7.37 (m, 7H, H-5/ H-7/ H-2"/ H-3"/ H-4"/ H-5" /H-6"), 8.33-8.38 (m, 1H, H-8)

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No.	Ar	X	-C*H*$_3$ (s, 3H)	**H-3'/H-5' (m, 4H)**	**H-2'/H-6' (m, 4H)**	**Other peaks**
42c	$C_6H_5CH_2$-	Cl	2.53	2.68-2.71	3.41-3.44	3.62 (s, 2H, C*H*$_2$), 7.25-7.39 (m, 5H, H-2"/ H-3"/ H-4"/ H-5"/ H-6"), 7.51 (dd, 1H, *J* = 9.0, 1.9, H-7), 7.84 (d, *J* = 1.9, 1H, H-5), 8.28 (d, *J* = 9.0, 1H, H-8)
42d	$C_6H_5CH_2$-	Br	2.52	2.68- 2.71	3.41- 3.44	3.62 (s, 2H, C*H*$_2$), 7.24-7.39 (m, 5H, H-2"/ H-3"/ H-4"/ H-5"/ H-6"), 7.64 (dd, 1H, *J* = 9.0, 1.9, H-7), 8.03 (d, *J* = 1.9, 1H, H-5), 8.20 (d, *J* = 9.0, 1H, H-8)
43a	*p*-C_6H_4F-	H	2.64	3.33-3.37	3.54-3.57	6.95-7.03 (m, 4H, H-2"/ H-3"/ H-5"/ H-6"), 7.63-7.66 (m, 2H, H-5/ H-7), 7.89-7.92 (m, 1H, H-6), 8.36-8.40 (m, 1H, H-8)
43b	*p*-C_6H_4F-	F	2.57	3.33- 3.36	3.55- 3.58	6.93-7.03 (m, 5H, H-2"/ H-3"/ H-4"/ H-5"/ H-6"), 7.37-7.46 (m, 2H, H-5/ H-7), 8.40 (dd, *J* = 9.2, 5.7, 1H, H-8)
43c	*p*-C_6H_4F-	Cl	2.59	3.33-3.36	3.55-3.58	6.91-7.03 (m, 4H, H-2"/ H-3"/ H-5"/ H-6"), 7.69 (d, *J* = 9.0, 1H,H-7), 8.08 (s, 1H, H-5), 8.69 (d, *J* = 9.0, 1H, H-8)
43d	*p*-C_6H_4F-	Br	2.57	3.32-3.35	3.54-3.57	6.92-7.02 (m, 4H, H-2"/ H-3"/ H-5"/ H-6"), 7,68 (dd, *J* = 9.0, 1.9, 1H, H-7), 8.06 (d, *J* = 1.9, 1H, H-5), 8.23 (d, *J* = 9.0, 1H, H-8)

Table 5. The chemical shift values (δ) of the different carbons of compounds (**41-43**)



No.	Ar	X	-*C*H_3	C-2'/C-6'	C-3'/C-5'	Other peaks
41a	C_6H_5-	H	12.6	49.5	50.9	116.2, 119.1, 122.2, 122.8, 127.9, 128.2, 129.1, 130.1, 130.3, 148.0, 151.4, 159.2
41b	C_6H_5-	F	13.0	49.6	50.9	105.7, 106.0, 116.3, 119.1, 119.5, 120.0, 121.2, 129.3, 129.8, 133.6, 133.8, 145.9, 151.5, 159.2, 161.2, 164.6
41c	C_6H_5-	Cl	17.7	49. 5	50.7	116.2, 119.9, 124.6, 127.3, 128.5, 129.1, 130.2, 131.5, 132.5, 149.1, 151.3, 160.7
41d	C_6H_5-	Br	12.8	49.5	50.9	116.2, 120.0, 120.5, 125.3, 125.6, 129.2, 131.6, 132.0, 132.7, 146.4, 151.4, 159.5
42a	$C_6H_5CH_2$-	H	12.8	50.9	53.5	63.3 (*C*H_2), 121.8, 123.0, 127.2, 127.8, 128.4 (2x), 129.3, 130.3, 132.9, 138.1, 148.0, 159.5
42b	$C_6H_5CH_2$-	F	13.0	50.6	53.3	63.2 (*C*H_2), 105.5, 105.8, 118.7, 119.1, 120.8, 120.9, 127.2, 128.3, 129.2, 129.8, 133.5, 133.6, 137.9, 145.7, 159.3, 161.0, 164.4

No.	Ar	X	$-CH_3$	C_2'/C_6'	C_3'/C_5'	Other peaks
42c	$C_6H_5CH_2$-	Cl	12.9	50.7	53.3	63.2 (*C*H_2), 120.3, 121.7, 127.1, 128.3, 128.9, 129.0, 129.2, 132.0, 136.6, 138.0, 146.1, 159.6
42d	$C_6H_5CH_2$-	Br	13.0	50.8	53.4	63.3 (*C*H_2), 120.2, 125.3, 125.5, 127.2, 128.4, 129.3, 129.5, 131.4, 132.0, 138.0, 146.3, 159.7
43a	*p*-C_6H_4F-	H	12.6	50.5	51.0	115.4, 115.7, 117.9, 118.0, 122.1, 122.9, 127.9, 128.2, 130.2, 130.3, 148.1 (2x), 155.7, 158.8, 159.1
43b	*p*-C_6H_4F-	F	13.1	50.6	50.9	105.7, 106.0, 115.5, 115.8 , 118.1, 118.2, 119.2, 119.6, 121.4, 133.6, 133.7, 145.9, 148.1, 153.7, 155.8, 159.0, 159.2, 161.3, 164.6
43c	*p*-C_6H_4F-	Cl	12.9	50.6	50.9	115.5, 115.7, 118.0, 118.1, 120.3, 121.8, 129.0, 129.2, 131.8, 136.8, 146.1, 147.5, 155.8, 158.9, 159.8
43d	*p*-C_6H_4F-	Br	12.8	50.5	50.8	115.4, 115.7, 117.9, 118.0, 120.5, 125.2, 125.6, 129.4, 131.6, 131.9, 146.4, 148.0, 155.7, 158.9, 159.4

4.3. Biological activity

4.3.1. Methods and results

4.3.1.1. Bacterial and Candida Cultures

The chemical compounds were tested for their activity against bacterial and Candida (fungi or yeast) strains using laboratory controls from American Type Culture Collection (ATCC) (Rockville, MD and U.S.A) and clinical isolates which were a gift from Basem Jaber (PhD of medical mycology, the University of Jordan, Faculty of Science, Department of Biology). The ATCC strains were *Staphylococcus aureus* ATCC 25923 (fully susceptible strain), methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Candida glabirata* ATCC 15126, *Candida albicans* clinical isolate (urinary tract infection), *Candida glabirata* clinical isolate 1 & 2 (neonate infections). Bacterial strains were cultured overnight at 37^{o}C in Mueller-Hinton broth and Candida strains were cultured overnight at 37^{o}C in Sabouraud Dextrose broth.

4.3.1.2. Compound Susceptibility Testing

4.3.1.2.1. Disk Diffusion Method / (Kirby Bauer Test)

The synthetic compound were tested *in vitro* for their antibacterial activity against gram positive (Staphylococcal strains) and gram negative organism (*E. coli*) and Candida at 0.025 g/ml by modified Kirby- Bauer agar diffusion method (Bauer AW. *et al.,* 2006)

The National Committee for Clinical Laboratory Standards (NCCLS) guidelines recommends using Mueller-Hinton agar medium for bacteria and Sabouraud dextrose agar medium for *Candida.*

Wells were punched in the agar plates (6 mm wide) and inoculated with different bacteria and Candida. The wells were filled with 100 μL of the tested compound and the plates were incubated at 37 ^{o}C for 24 hours. The diameters of the inhibition zones were measured in millimeters (mm). Each antimicrobial assay was performed in triplicates and mean values were reported. Standard antibiotics, gentamycin (10 μg/disc), and nystatin (25 μg/disc) served as positive controls for antimicrobial and Candida activity, respectively.

Solvent control wells of dimethyl sulfoxide (100 μL of DMSO) were used to aid in solubilizing nystatin and they were used as negative control. The inhibition zone diameters were measured. The organisms used and zone of inhibition to the corresponding compounds are shown in Table 5.

4.3.1.2.2. Serial Dilution Method (Broth Microdilution Assay)

According to NCCLS, a broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/ fungicidal concentration (MBC/MFC).

The MIC of a compound was defined as the lowest concentration of the compound that resulted in complete inhibition of visible bacterial /fungal growth at 24 h.

The inocula of the bacterial strains or Candida were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. A serial doubling dilution of the compounds was prepared in a 96/well microtiter plate. A double strength of Mueller Hinton broth/ Sabouraud Dextrose broth was used as a diluent. The concentrations were in range of 0.025-0.00002 g/mL. Bacterial strains and Candida were inoculated in Mueller Hinton broth and Sabouraud dextrose broth respectively and inoculated into wells (the final concentration in each well adjusted to 2.0 x 10^6 CFU/mL for bacteria and 2.0 x

10^5 of Candida strains). The plate was incubated for 24 h at 37°C. A control well containing the growth medium and the bacteria or Candida was set-up. Gentamicin and nystatin served as positive controls, while the solvent (DMSO) was used as a negative control. MIC was defined as the lowest concentration of compound that resulted in complete inhibition of visible (turbidity in the broth) bacterial / fungal growth at 24 h. To determine MBC / MFC broth was taken from each well and inoculated in Mueller Hinton agar for 24 h at 37°C for bacteria or in Sabouraud dextrose agar for Candida strains respectively.

The MBC/MFC were defined as the lowest concentration of the compound that kills 99.9% of the original inoculum in 24 h. Tables (5, 6, 7 & 8) and figures (2, 3 & 4) show the MIC and MBC/MFC to the corresponding compounds and the organisms used.

4.3.2. Results

Table 5: Antibacterial and antifungal activity of various chemical compounds

Compound 25 µg/ml	*S. aureus ATCC 25923*	*MRSA ATCC 33591*	*E. coli ATCC 8739*	*C. albicans ATCC 10231*	*C. glabirata ATCC 15126*	*C. albicans Clinical isolate*	*C. glabirata Clinical isolate 1*	*C. glabirata Clinical isolate 2*
41a	NA	NA	NA	NA	8 ± 0.5	7 ± 0.5	NA	NA
41d	NA	NA	NA	NA	7 ± 0.5	7 ± 0.1	NA	NA
41b	NA	NA	NA	NA	7 ± 0.1	7 ± 0.1	NA	NA
41c	NA	NA	NA	NA	7 ± 0.8	7 ± 0.5	NA	NA
42a	NA	NA	7 ± 0.7	7 ± 0.2	NA	8 ± 0.6	NA	NA
42d	NA	NA	NA	7 ± 0.2	NA	7 ± 0.5	NA	NA
42b	NA	NA	NA	7 ± 0.2	NA	7 ± 0.2	NA	NA
42c	NA	NA	NA	NA	NA	7 ± 0.6	NA	NA
43a	NA	NA	NA	8 ± 0.4	8 ± 0.5	8 ± 0.3	NA	NA
43d	NA	NA	NA	7 ± 0.5	9 ± 0.3	8 ± 0.2	NA	NA
43b	NA	NA	NA	NA	8 ± 0.7	7 ± 0.1	NA	NA
43c	NA	NA	NA	NA	8 ± 0.4	7 ± 0.1	NA	NA
Gentamycin (10 µg/ml)	23 ± 1	21 ± 0.6	26 ± 1	-	-	-	-	-
Nystatin 25 µg/ml	-	-	-	17 ± 1.5	20 ± 0.5	15 ± 1	18 ± 1	18 ± 0.5

The results are the mean ± SD (n=3). The well is 6 mm wide. NA: Not active at 0.02g/ml of the compound tested.
Gentamicin and Nystatin used are disks not in wells. Disks are 5 mm wide.

At first stage compounds (25 µg/mL) in wells were used and showed no antibacterial activity either against gram positive *S. aureus* strains (ATCC 25923 and MRSA 33591) and gram negative *E. coli* (ATCC 8739) or *Candida glabirata* clinical isolate 1 and 2.

Table 6: The MIC and MFC in g/mL for compounds (43a-d) against *Candida* species

	43a		**43b**		**43c**		**43d**		**Nystatin**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
C.albicans ATCC 10231	**0.0003**	**0.0006**	**NA**	**NA**	**NA**	**NA**	**0.0005**	**0.0009**	**0.000006**	**0.00003**
C. albicans Clinical isolate	**0.0003**	**0.0006**	**0.0003**	**0.0006**	**0.0006**	**0.001**	**0.0002**	**0.0005**	**0.000008**	**0.00003**
C.galibrata1	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**0.000004**	**0.00002**
C.galibrata2	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**0.000004**	**0.00002**
C .galibrata ATCC 15126	**0.0003**	**0.0006**	**0.0002**	**0.0005**	**0.0006**	**0.0006**	**0.0002**	**0.0002**	**0.000003**	**0.00002**

NA: No activity

The compounds **41a-d** and **42a-d** showed no activity against *C. glabirata* ATCC and *C. albicans* ATCC, respectively.

However; a fairly good activity for the compounds was found when tested against *C. albicans* clinical isolates.

The results of the broth microdilution assay for **43c** and **43d** showed that the two compounds had fungicidal effect against *C. galibirata* ATCC strain.

Furthermore, **43b** and **43c** had no activity against *C. albicans* ATCC strains. The MIC and MFC for **43a** against *C. albicans* strains and *C. galibarata* ATCC strain were 0.0003 g/ml and 0.0006 g/mL, respectively.

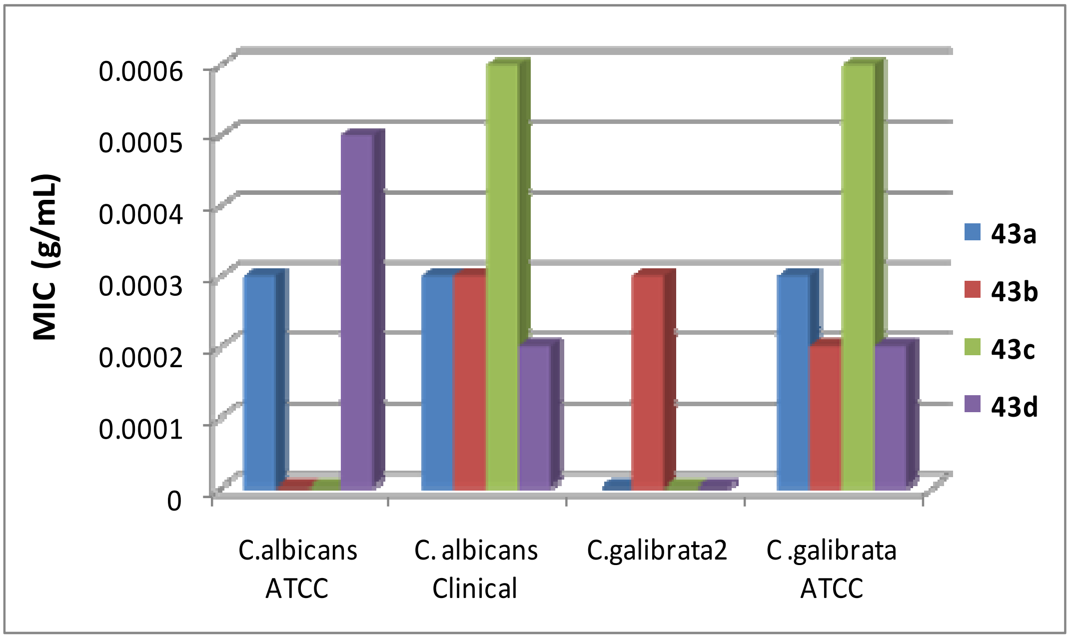


Figure 2: Antifungal activity for compounds (**43a-d**) against different Candida Strains

The results in figure 2 comparing the MIC for different compound against different Candida strains showed that the **43a** MIC range was (not active – 0.003), the compound **43d** MIC was (0.0005-0.002 g/mL), **43b** MIC was (not active- 0.0003 g/mL) and **43c** MIC was (not active- 0.0006 g/mL). The compounds **43a-d** are found to have species-specific effect and species-specific MIC concentrations.

Table 7: The MIC and MFC in g/mL for compounds (41a-d) against *Candida* species

	41a		41b		41c		41d		Nystatin	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C .albicans ATCC 10231	NA	NA	NA	NA	NA	NA	NA	NA	0.000006	0.00003
C.albicans clinical isolate	0.0004	0.0009	0.0003	0.0006	0.0004	0.0009	0.003	0.005	0.000008	0.00003
C.galibrata1	NA	NA	NA	NA	NA	NA	NA	NA	0.000004	0.00002
C.galibrata2	NA	NA	NA	NA	NA	NA	NA	NA	0.000004	0.00002
C.galibrata ATCC 15126	0.0004	0.0004	0.0002	0.0002	0.0004	0.0004	0.003	0.003	0.000003	0.00002

NA: No activity

The results in table 7 showed that **41a-d** did not have any activity against *C. albicans* ATCC strain and *C. galibrata* clinical isolates 1 & 2. The compounds **41a-d** showed fungicidal rather than fungistatic in range of (0.0002-0.003 g/ml) against *C. galibrata* ATCC strain. The **41a-d** compounds had MIC ranged (0.0003-0.005 g/mL) against C. *albicans* clinical isolates.

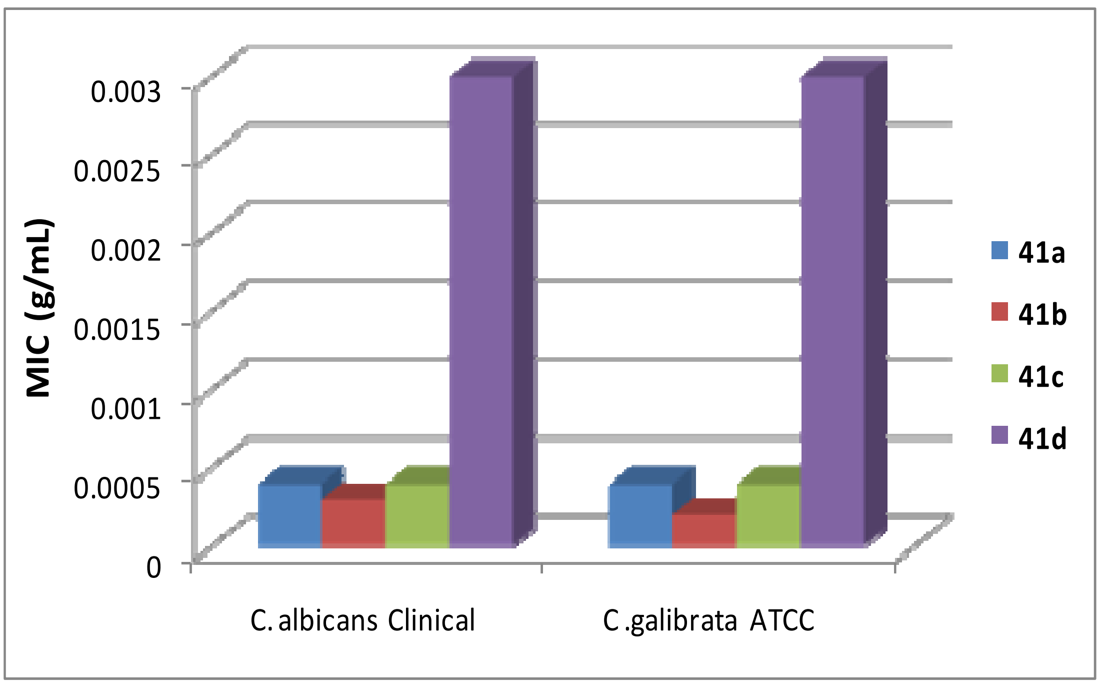


Figure 3: Antifungal activity for compounds (**41a-d**) against different Candida Strains

The results in figure 3 showed that **41a**, **41c** and **41d** are more effective with no significant difference against *C. albicans* clinical isolate and *C. galibrata* ATCC strains when compared to **41d** with MIC 0.003 g/ml against the same strains. This indicates that **41a-d** compounds except **41d** are good antifungal compounds.

Table 8: The MIC and MFC in g/mL for compounds (42a-d) against *Candida* species

	42a		42b		42c		42d		Nystatin	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C .albicans ATCC 10231	0.0006	0.001	0.0006	0.001	NA	NA	0.0008	0.002	0.000006	0.00003
C. albicans clinical isolate	0.0002	0.0003	0.0006	0.001	0.00008	0.0002	0.0004	0.0008	0.000008	0.00003
C.galibrata1	NA	NA	NA	NA	NA	NA	NA	NA	0.000004	0.00002
C.galibrata2	NA	NA	NA	NA	NA	NA	NA	NA	0.000004	0.00002
C.galibrata ATCC 15126	0.0003	0.0003	0.0006	0.001	0.00016	0.0003	0.0004	0.0004	0.000003	0.00002

NA: No activity

Table 8 shows that **42a-d** compounds had no antifungal activity against *C. galibrata* clinical isolates (1 & 2) and *C. albicans* ATCC had no susceptibility to **42c**. Table 8 shows as well that **42a** and **42d** had fungicidal activity against *C. galibrata* ATCC strain. The MIC/MFC for **42a** ranges were (0.0002-0.0006 g/mL) /(0.0003-0.001) g/mL, the MIC /MFC for **42d** were (0.0004-0.0008 g/mL) /(0.0004-0.002) g/mL. Furthermore, the MIC /MFC for **42b** were 0.0006 g/mL / 0.001 g/mL and MIC/MFC for **42c** were (0.00008-0.00016 g/mL) / (0.0002-0.0003 g/mL).

The substance **42a** showed bacteriocidal activity against *E. coli* ATCC strain with MBC of 0.001g/mL (data not shown in table 8).

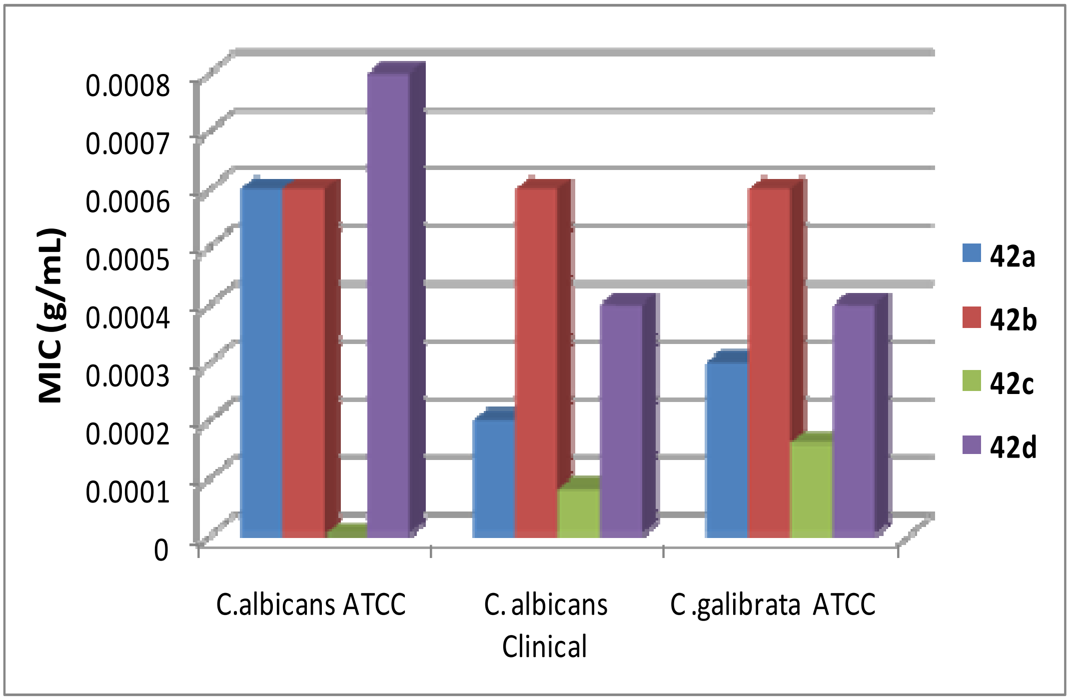


Figure 4: Antifungal activity for compounds (**42a-d**) against different Candida Strains

Results in Figure 4 indicated that the **42b** had antifungal activity against *C. albicans* strains (ATCC and clinical isolate) and *C. galibrata* ATCC strain only at MIC of 0.0006 g/mL. The compound **42a** MIC range was (0.0002-0.0006 g/mL) against the same Candida strains mentioned above. The antifungal activity for **42d** showed MIC range (0.0004-0.0008) against same Candida strains. Furthermore, *C. albicans* clinical isolate and *C. galibrata* ATCC strain were susceptible to **42c** MIC of about 0.00008 g/mL and 0.00016 g/mL, respectively.

4.3.2. Statistical Analyses

Analysis of variance (ANOVA) was used to determine the significance ($p \leq 0.05$) of the data obtained in all experiments. All results were determined to be within the 95% confidence level for reproducibility.

4.3.3. Discussion

In this study, a human pathogenic microorganisms and yeast (fungi) were examined, including Gram-positive (*S. aureus* ATCC and MRSA strains) and Gram-negative bacteria including *E. coli* ATCC and *Candida albicans* and *C. galibrata* clinical and ATCC starins.

Results indicated that all the compounds included in this study had no antibacterial effect except one **42a** had fungicidal effect against *E. coli* ATCC strain.

Susceptibility to compounds varied among *C. albicans* and *C.galibrata* clinical and ATCC strains. The tested compounds had no activity against *C. galibrata* clinical isolates (1&2) with antifungal activity against *C. albicans* clinical isolate.

The compounds **42a-d** showed no activity against *C. glabirata* ATCC but antifungal effect against *C. albicans* ATCC except for **42c**.

Compounds **43a-d** had higher activity against *C. glabirata* ATCC strain than *C. albicans* ATCC. However, **42a-d** compounds had higher activity against *C. albicans* ATCC than *C. glabirata* ATCC. Furthermore, **41a-d** compounds had better activity against *C. glabirata* than *C. albicans ATCC*.

Collectively, **43a-d** compounds had the highest activity against Candida strains then **41a-d** compounds and then **42a-d** compounds with the least activity.

Some of the tested compounds had fungicidal activity rather than fungistatic effect such as **43d**, **43c** and **41a-d**. However, only **42a** had bacteriocidal activity against *E. coli* ATCC strain with MBC of 0.001g/mL.

From the above studies it can be concluded that the compounds showed species-specific effect and species-specific MIC concentrations. The MBC were 2 to 3 fold higher than MIC values with few compounds were exclusively fungicidal (MIC=MBC).

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7. Appendix: NMR spectra

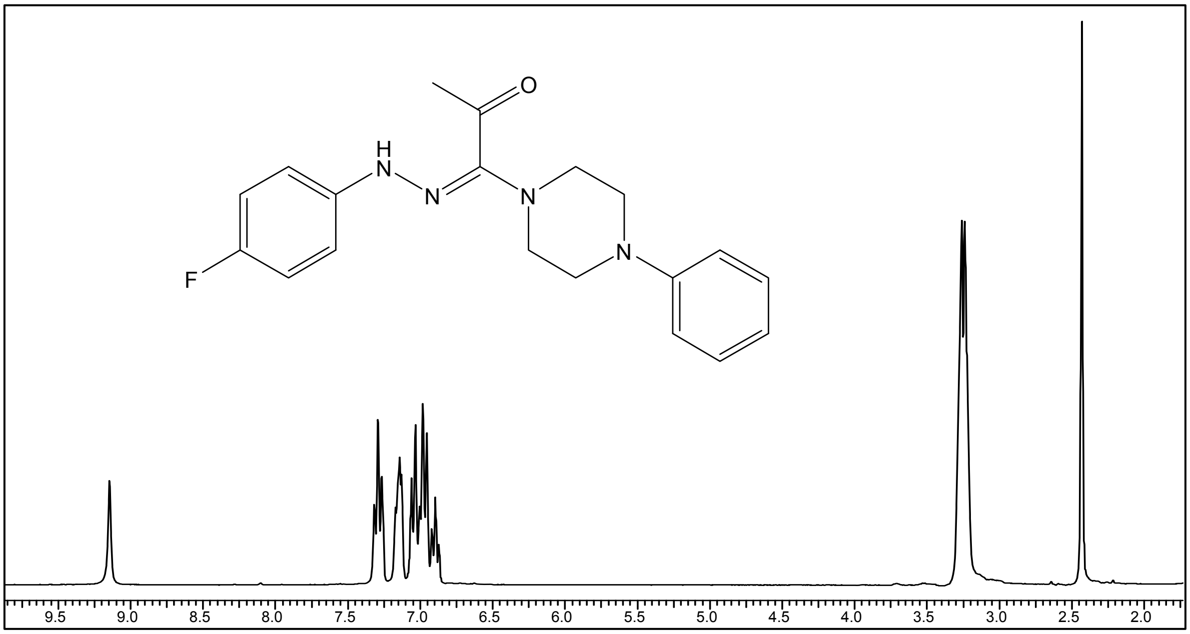


Figure 5: [1]H-NMR spectrum of compound [**38b**] ($CDCl_3$)

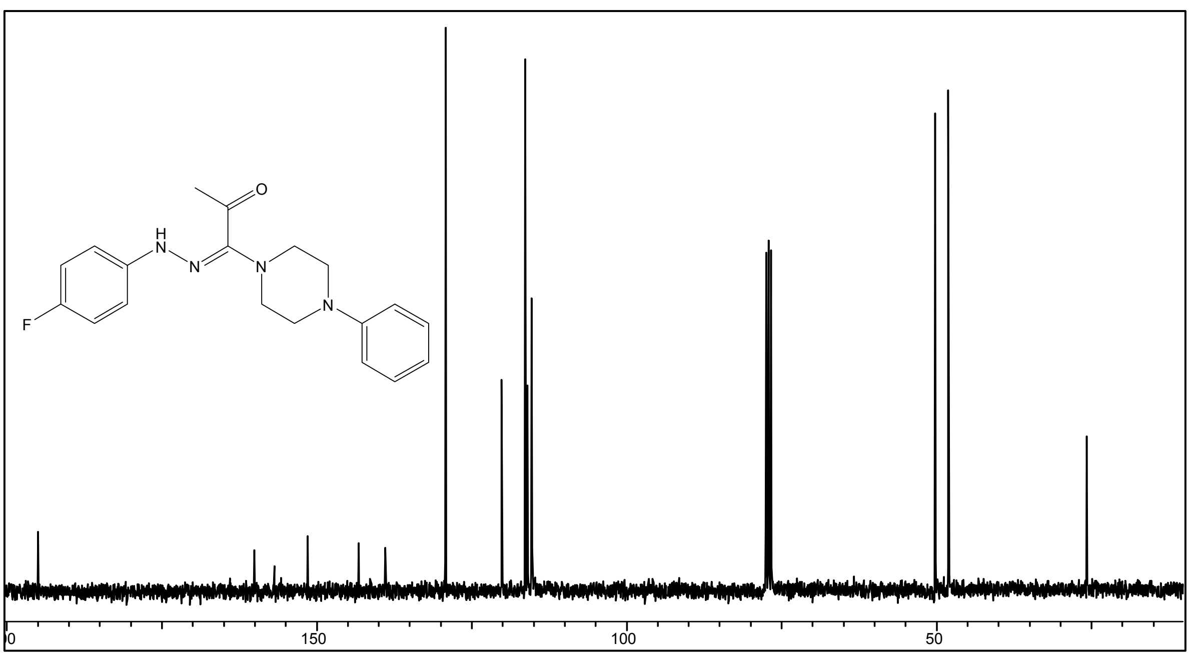


Figure 6: 13C-NMR spectrum of compound [**38b**] (CDCl3)

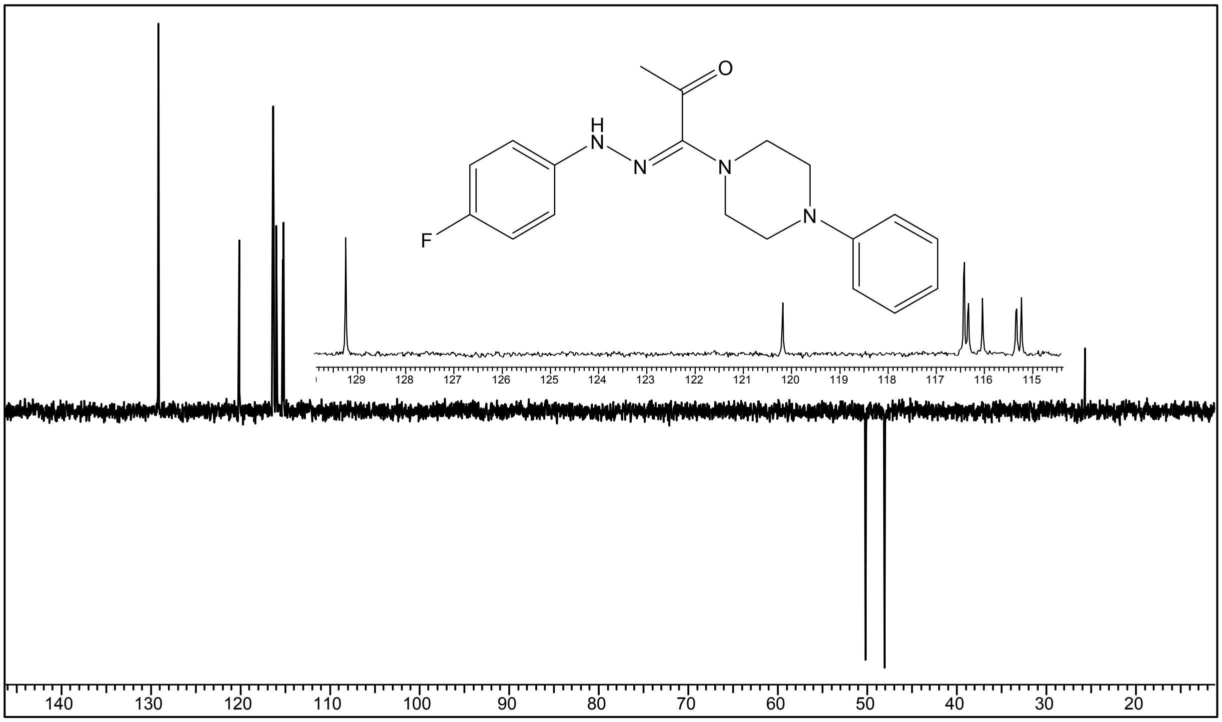


Figure 7: Dept 135 spectrum of compound [**38b**] (CDCl3)

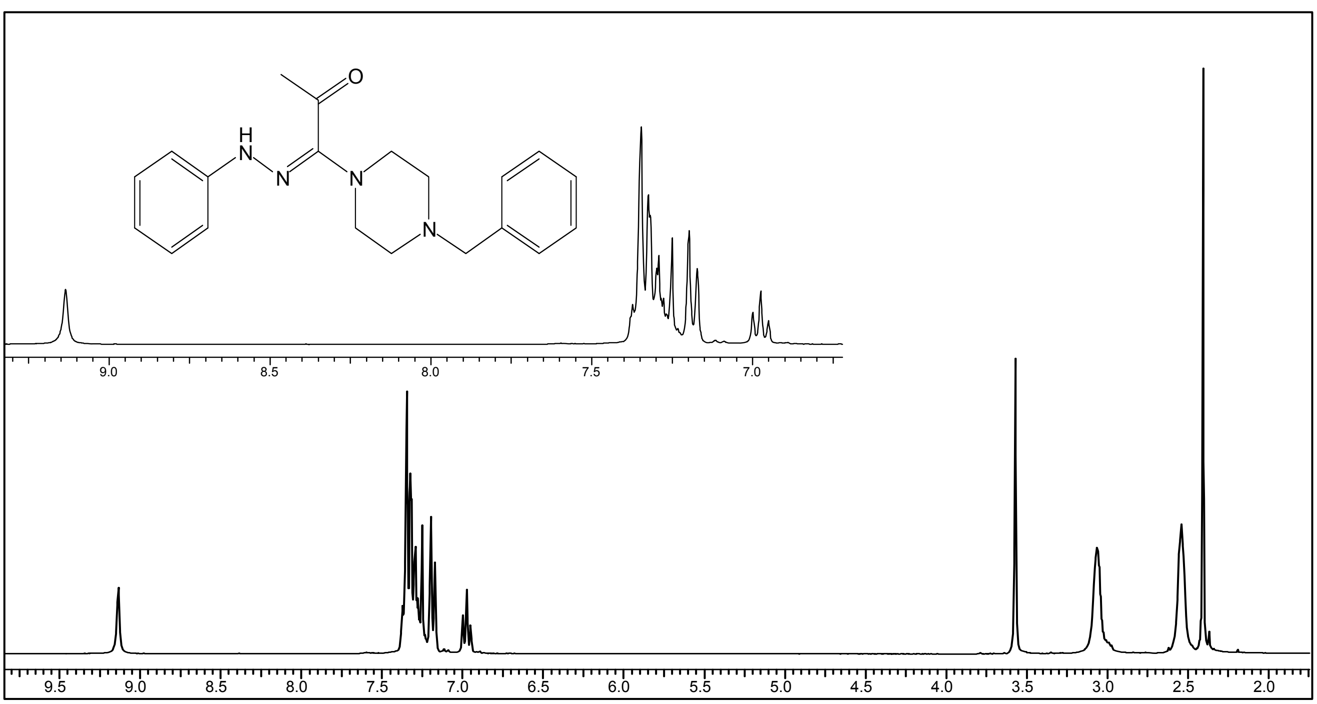


Figure 8: 1H-NMR spectrum of compound [**39a**] (CDCl3)

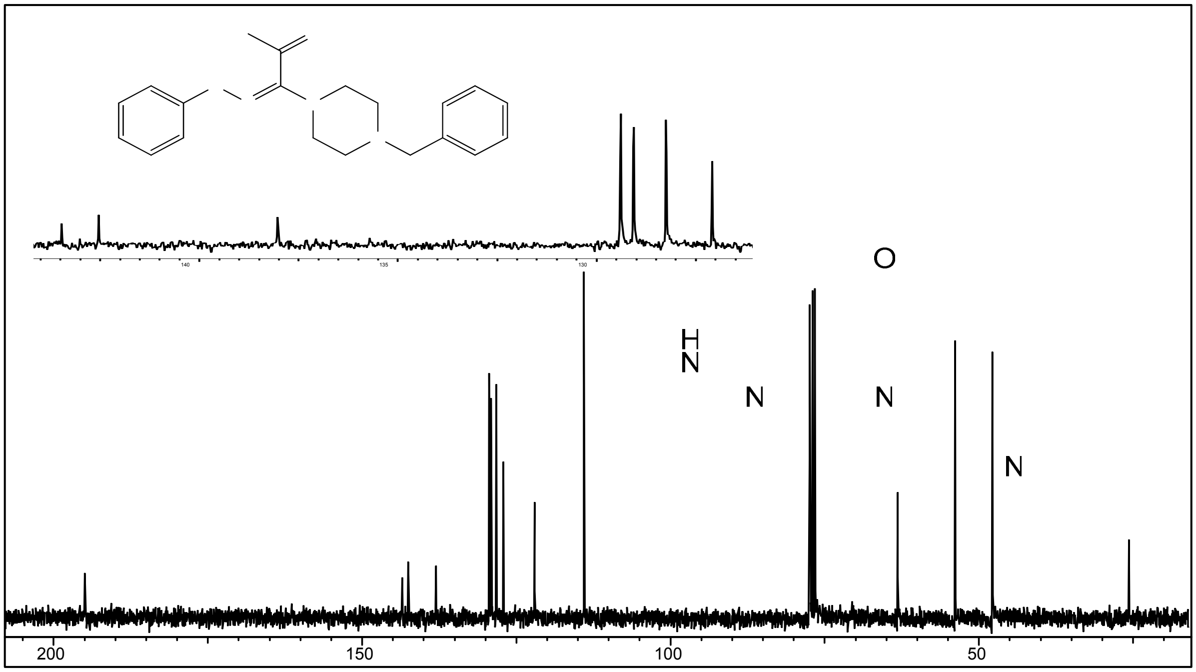


Figure 9: 13C-NMR spectrum of compound [**39a**] (CDCl3)

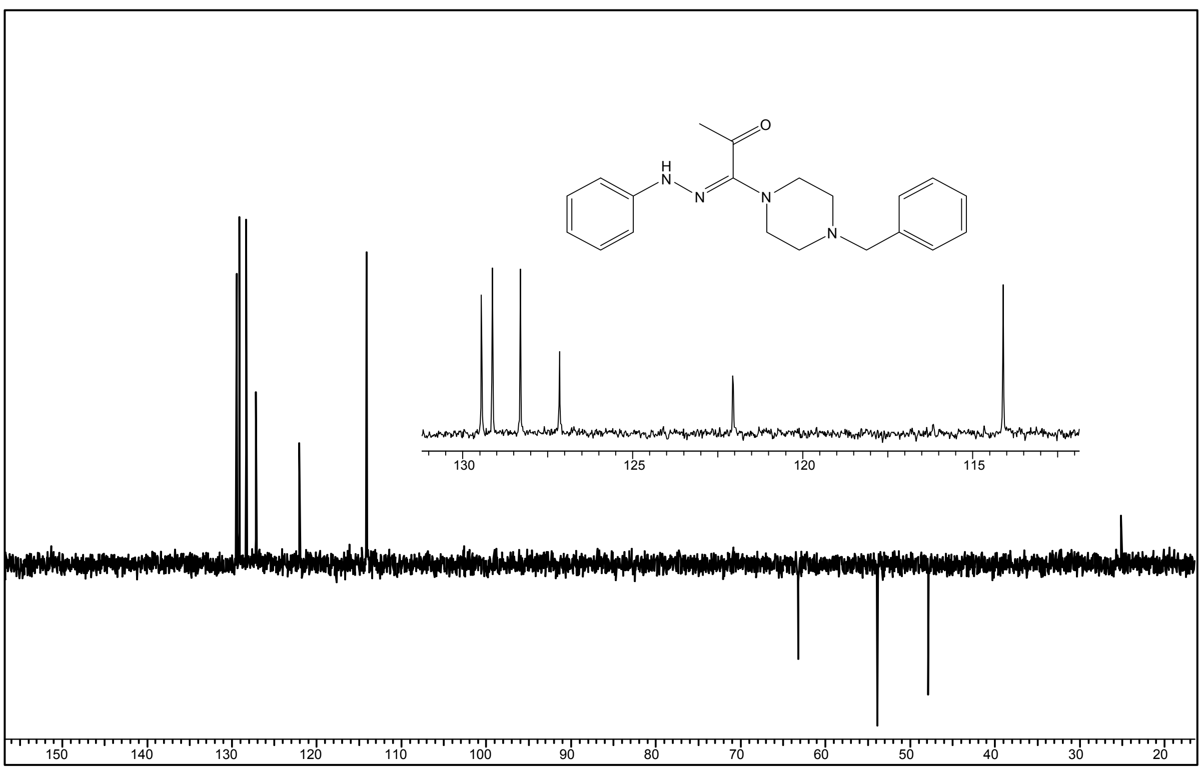


Figure 10: Dept 135 spectrum of compound [**39a**] (CDCl3)

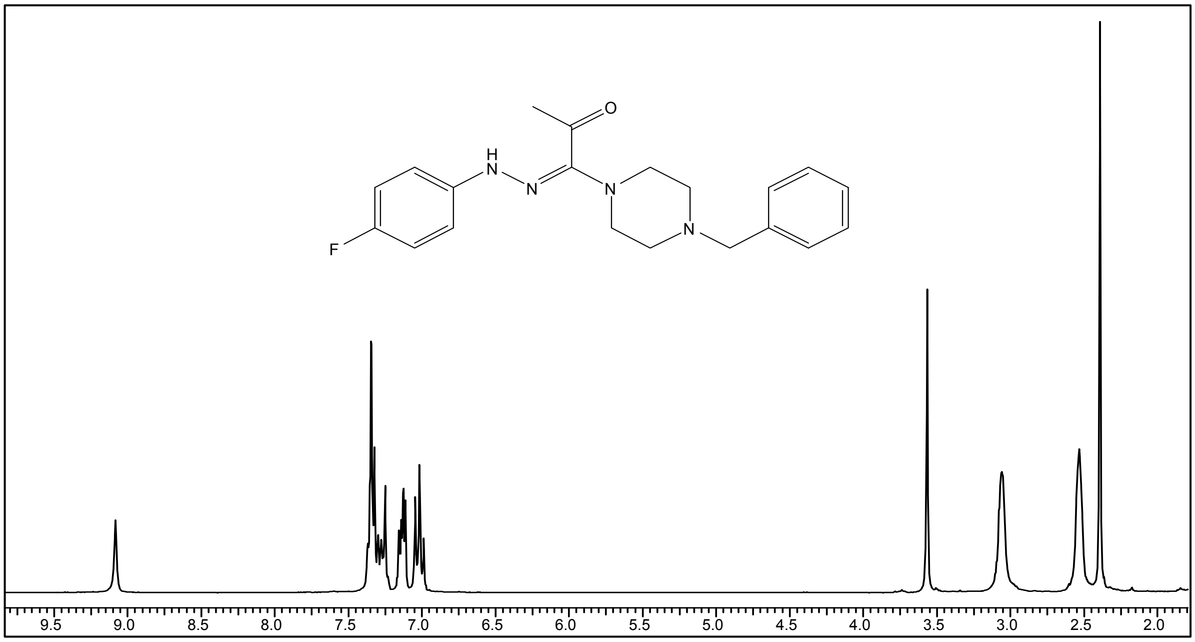


Figure 11: 1H-NMR spectrum of compound [**39b**] (CDCl3)

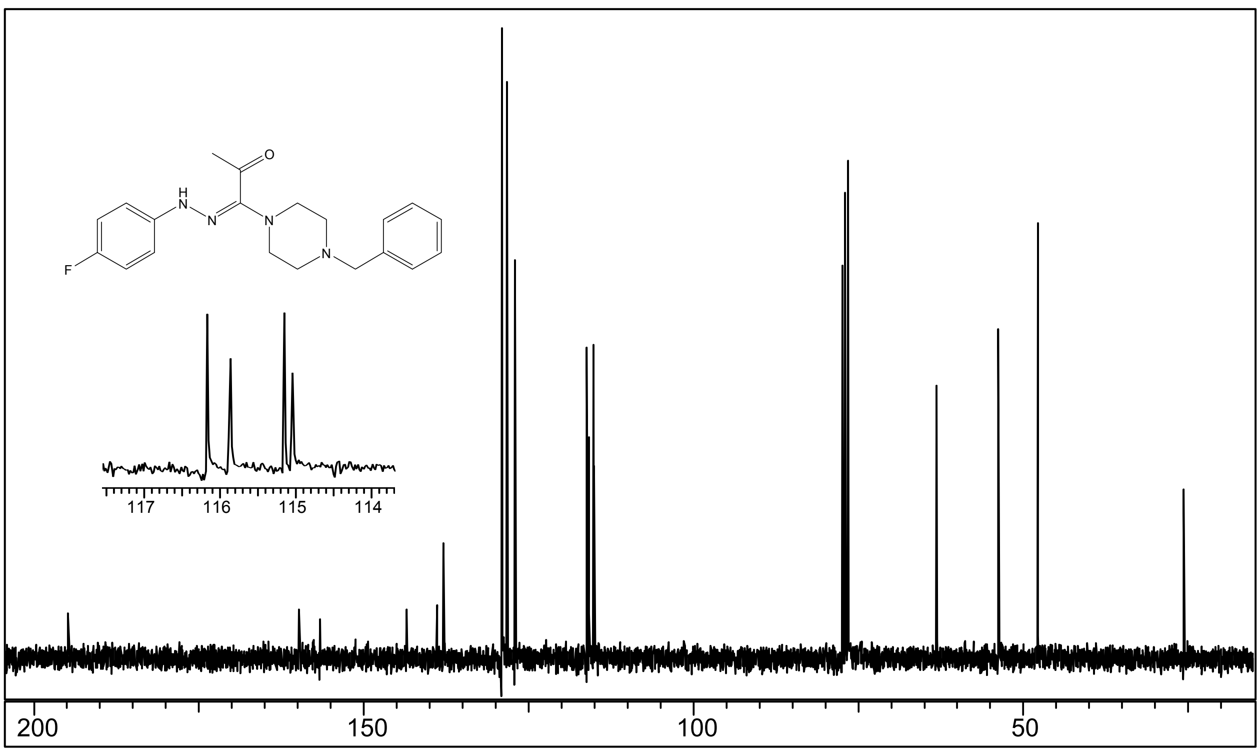


Figure 12: 13C-NMR spectrum of compound [**39b**] (CDCl3)

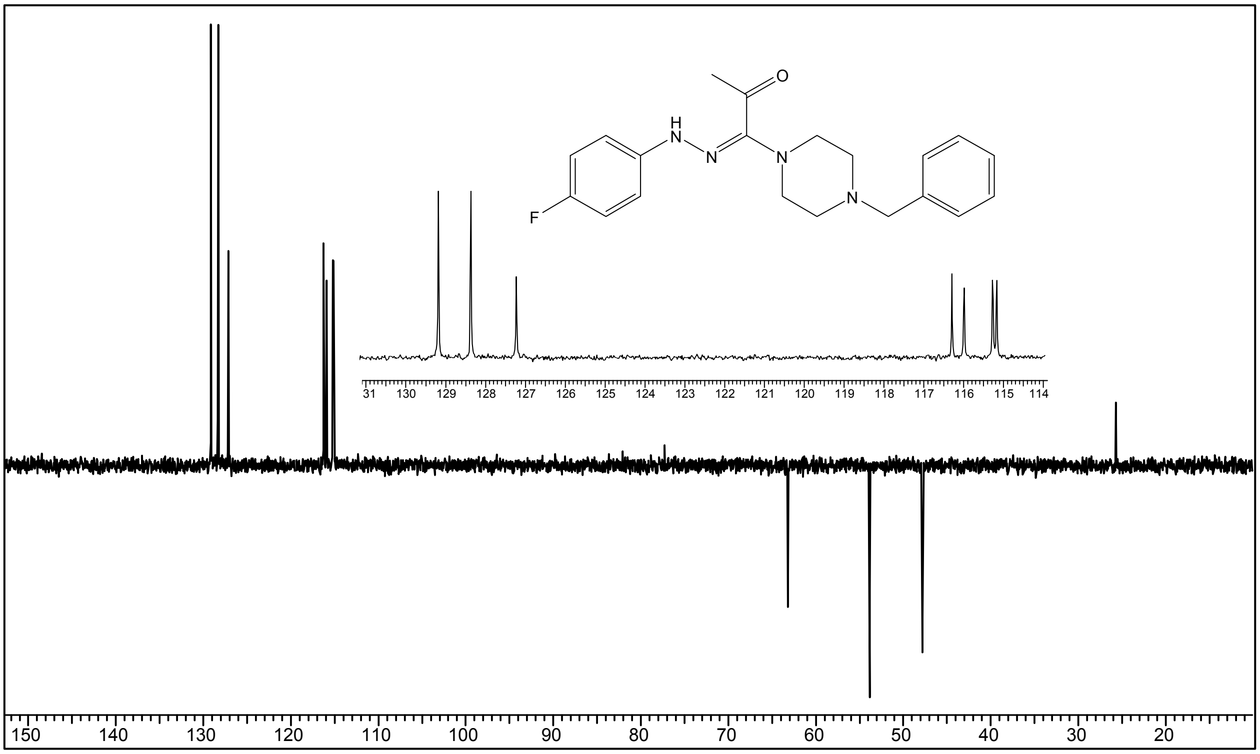


Figure 13: Dept 135 spectrum of compound [**39b**] ($CDCl_3$)

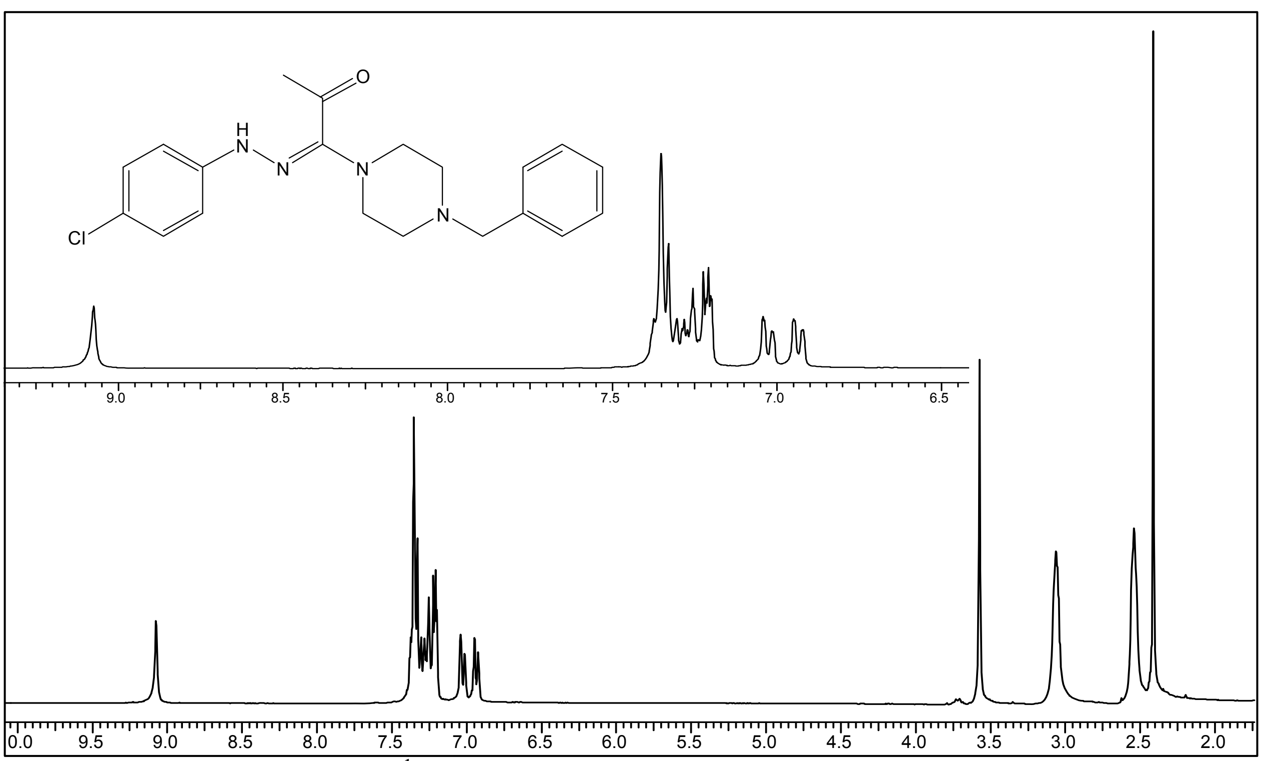


Figure 14: ^{1}H-NMR spectrum of compound [**39c**] ($CDCl_3$)

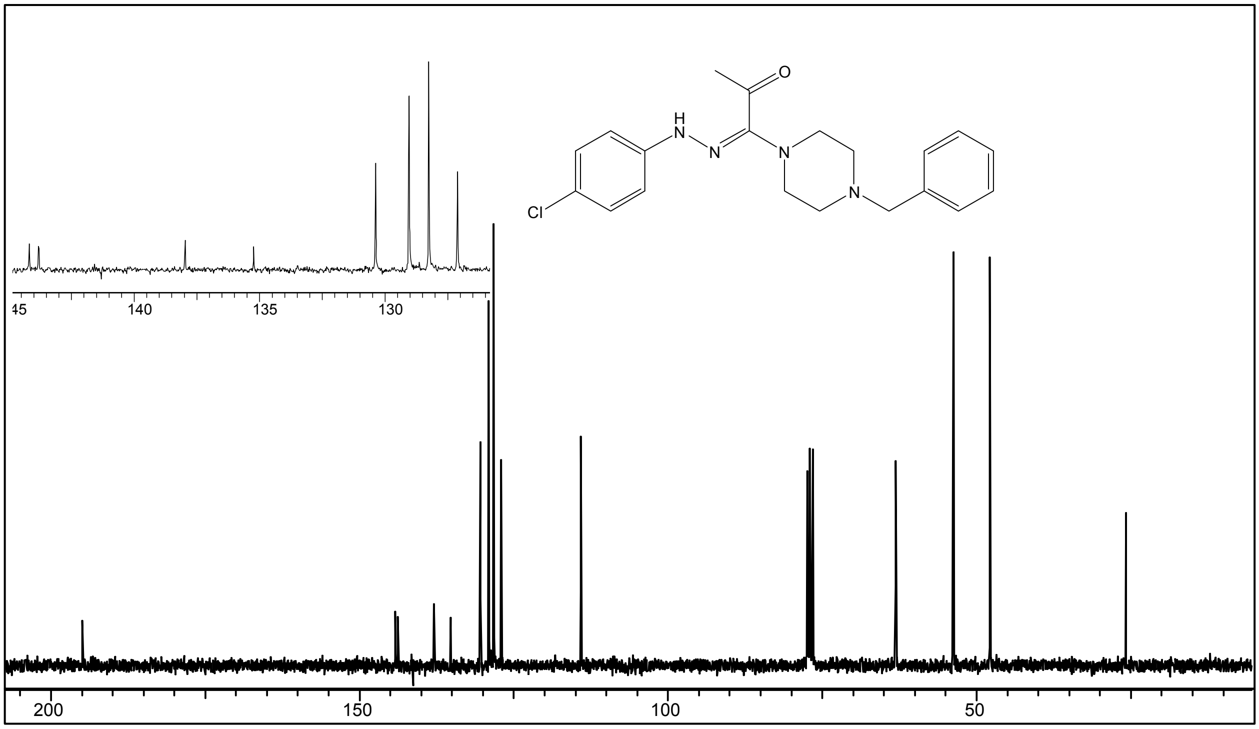


Figure 15: ^{13}C-NMR spectrum of compound [**39c**] ($CDCl_3$)

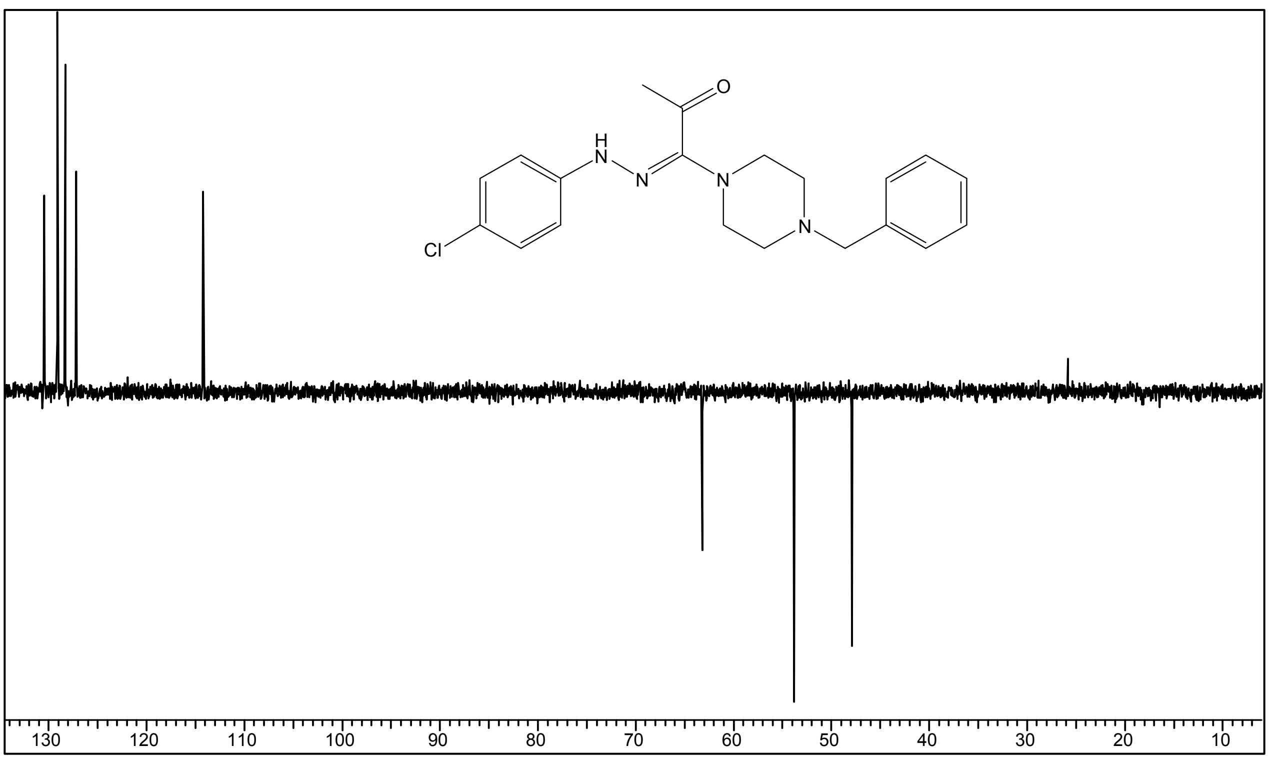


Figure 16: Dept 135 spectrum of compound [**39c**] ($CDCl_3$)

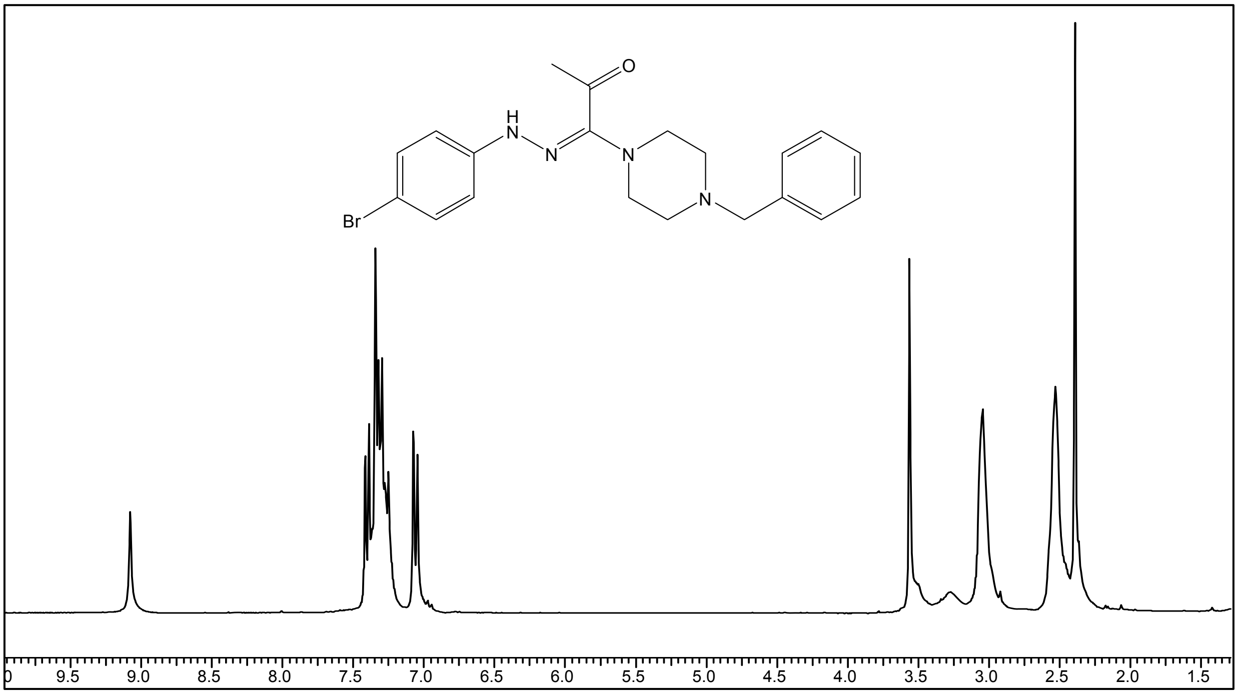


Figure 17: ^{1}H-NMR spectrum of compound [**39d**] ($CDCl_3$)

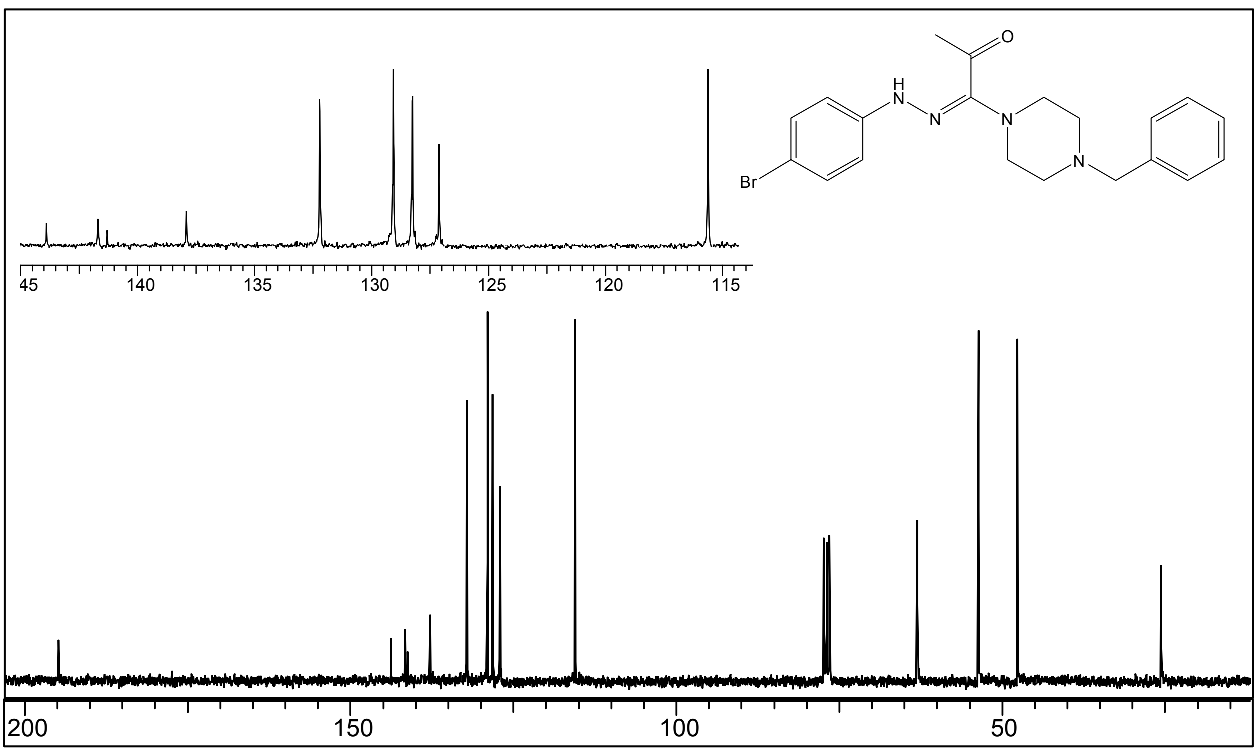


Figure 18: ^{13}C-NMR spectrum of compound [**39d**] ($CDCl_3$)

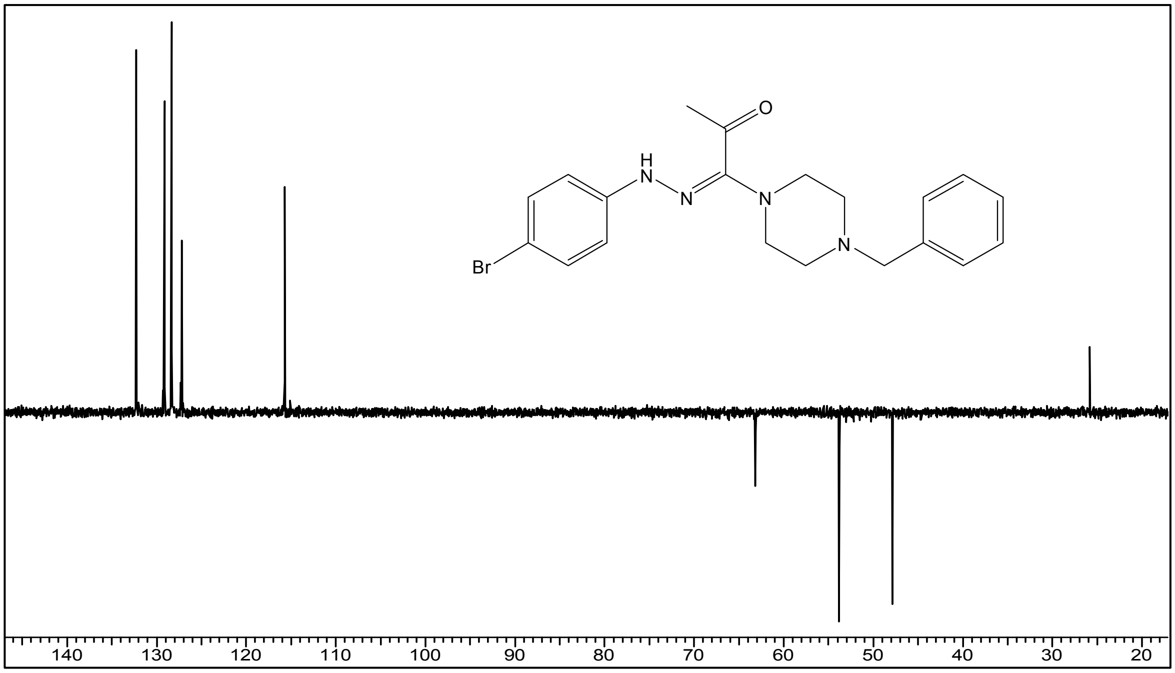


Figure 19: Dept 135 spectrum of compound [**39d**] ($CDCl_3$)

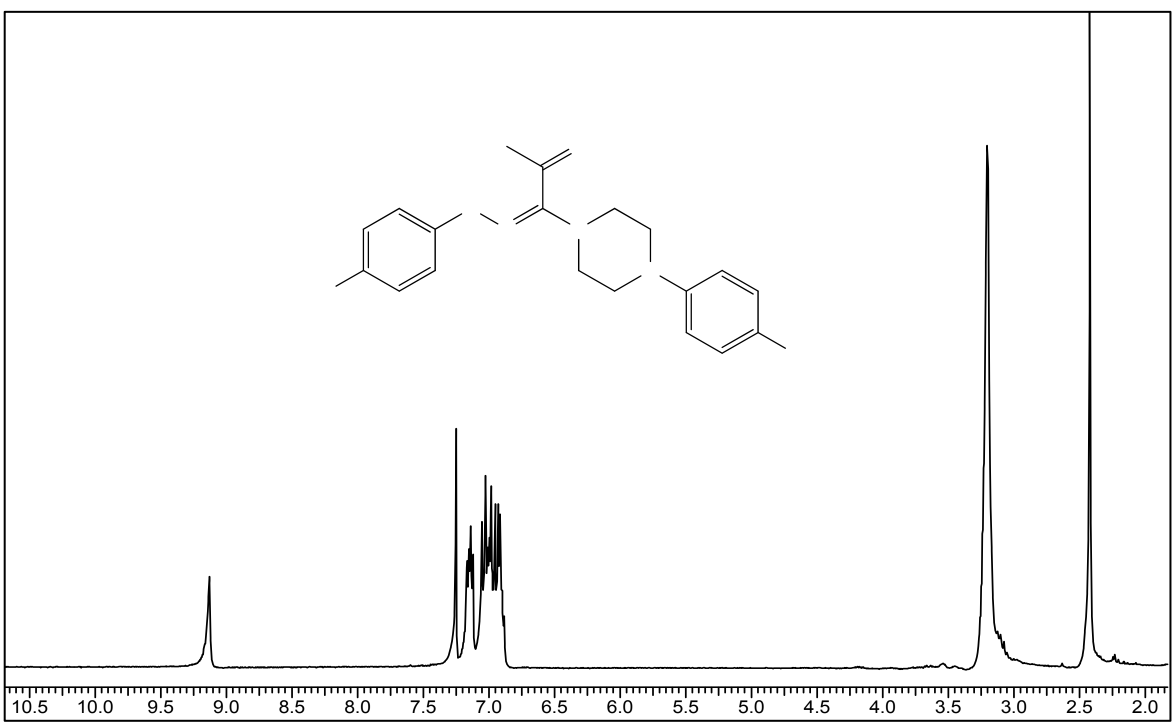


Figure 20: ^{1}H-NMR spectrum of compound [**40b**] ($CDCl_3$)

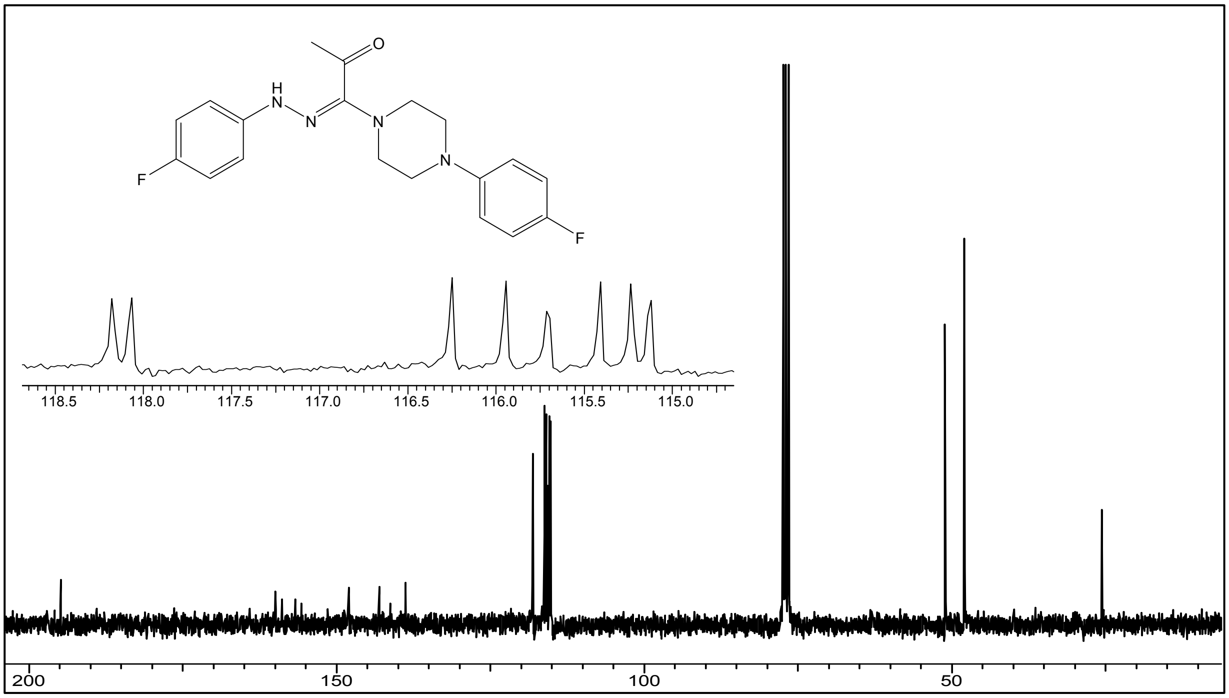


Figure 21: ^{13}C-NMR spectrum of compound [**40b**] ($CDCl_3$)

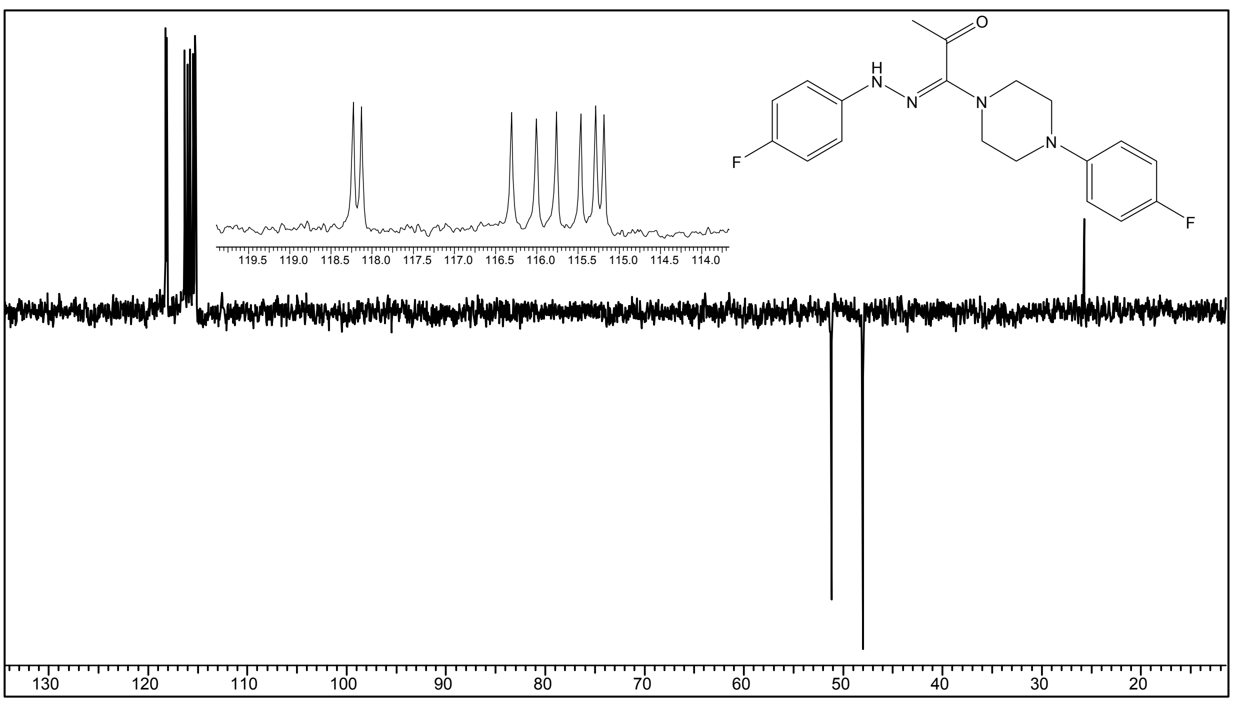


Figure 22: Dept 135 spectrum of compound [**40b**] ($CDCl_3$)

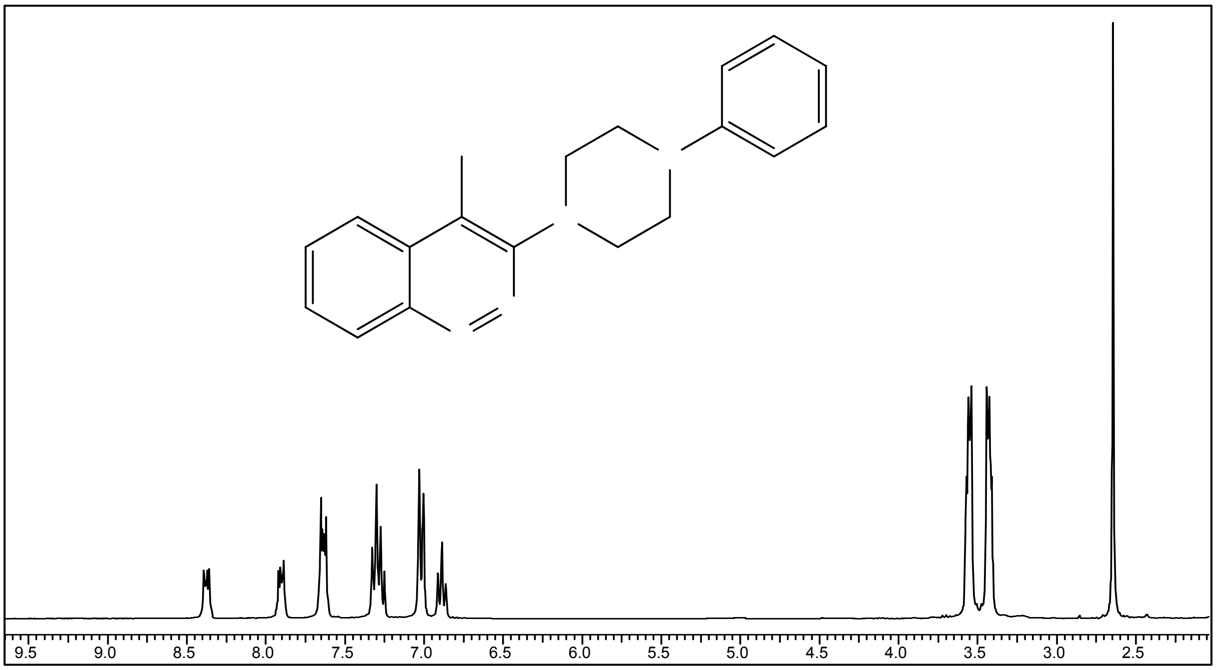


Figure 23: ^{1}H-NMR spectrum of compound [**41a**] ($CDCl_3$)

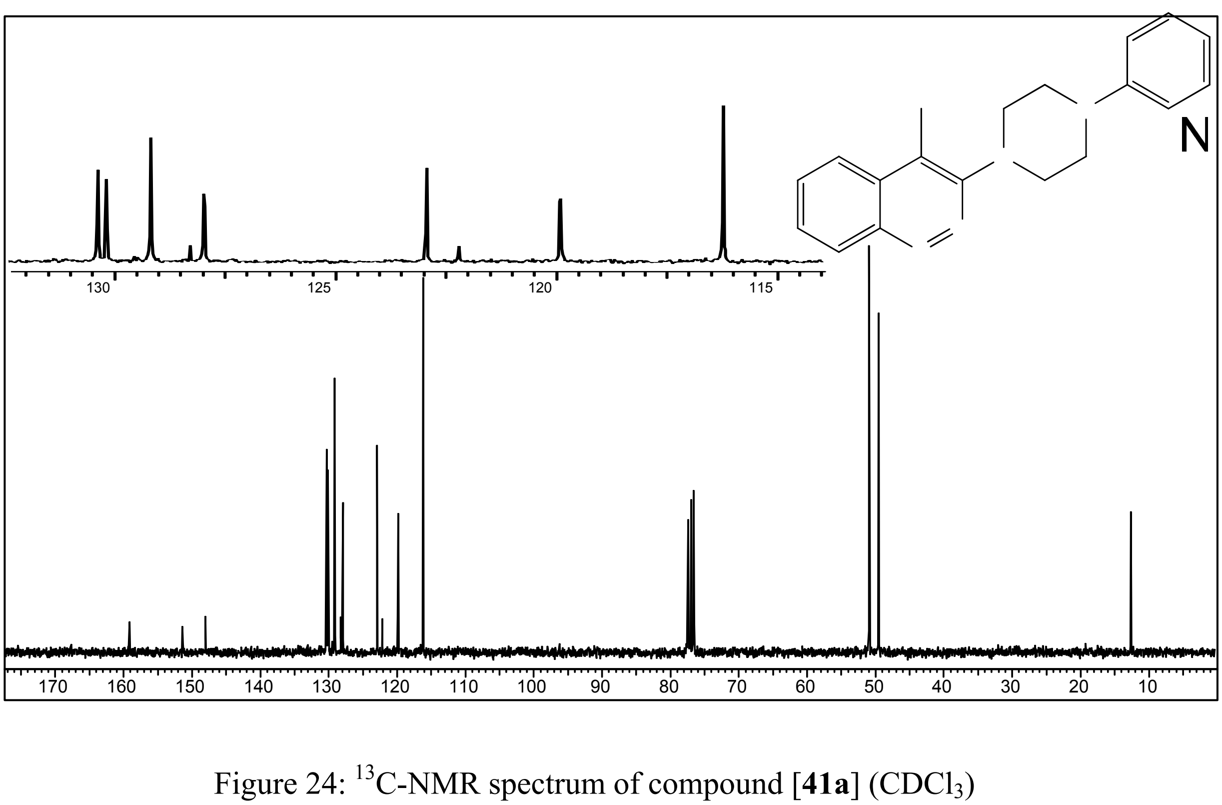


Figure 24: ^{13}C-NMR spectrum of compound [**41a**] ($CDCl_3$)

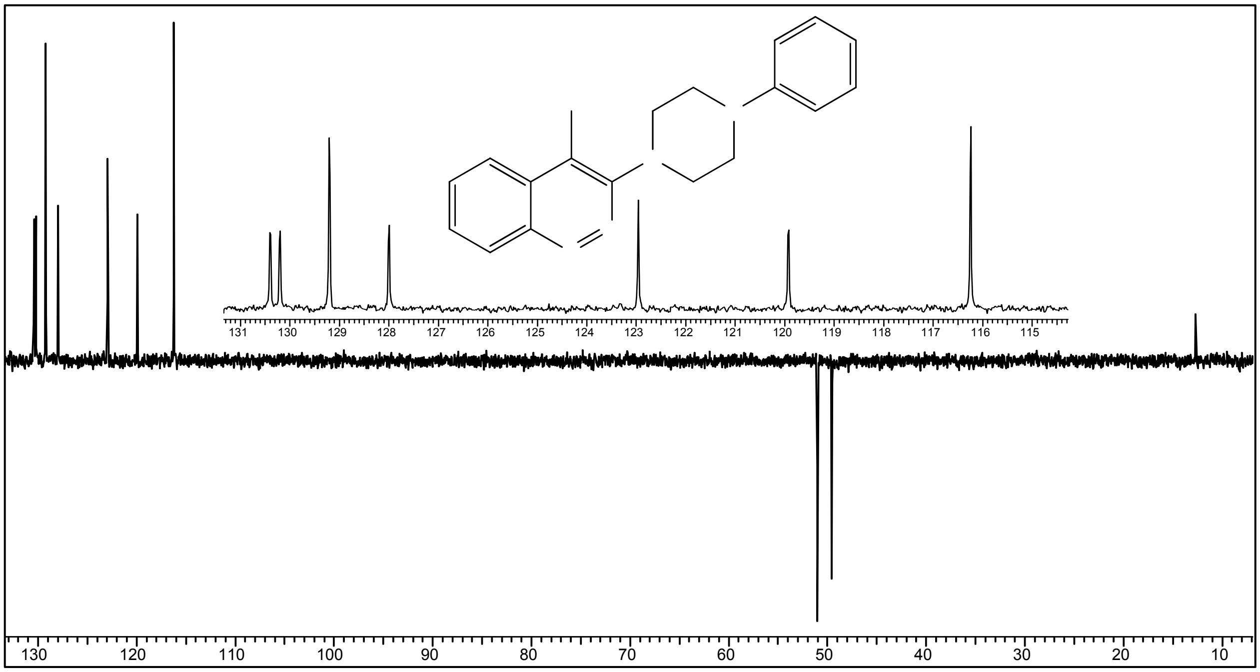


Figure 25: Dept 135 spectrum of compound [**41a**] ($CDCl_3$)

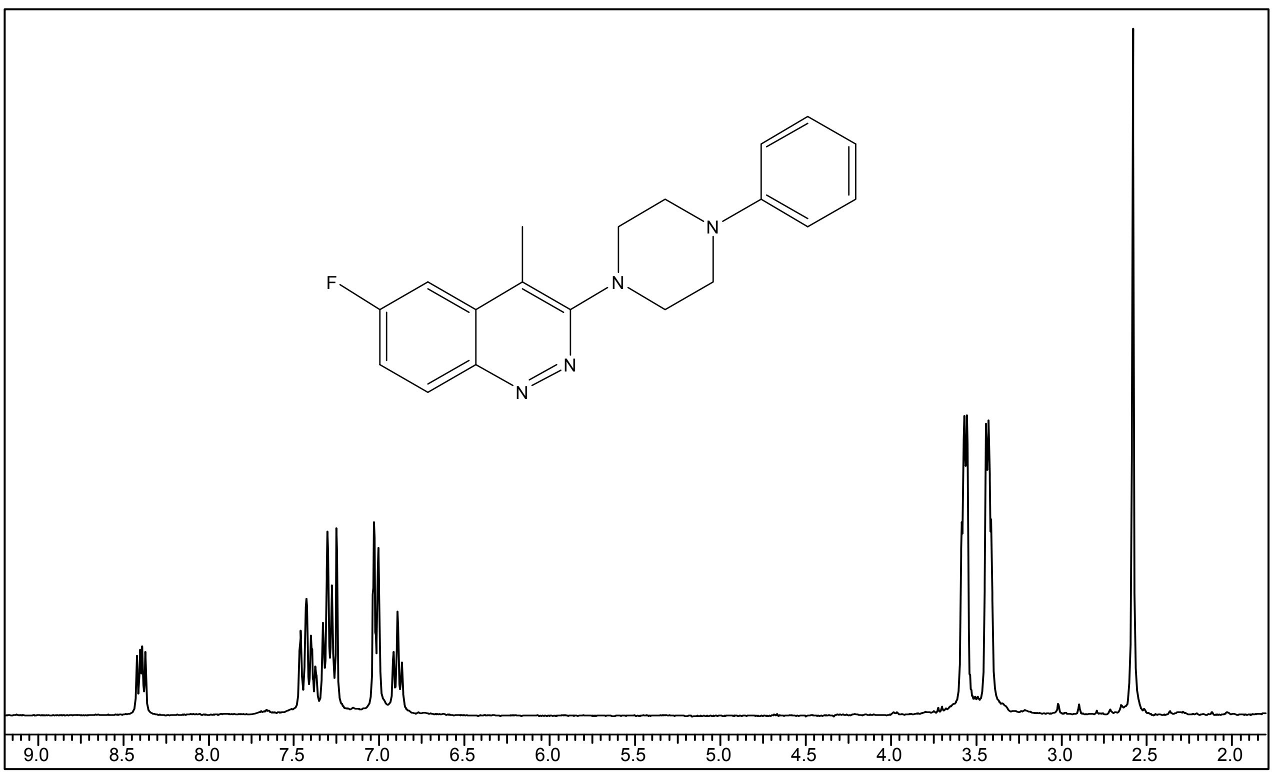


Figure 26: ^{1}H-NMR spectrum of compound [**41b**] ($CDCl_3$)

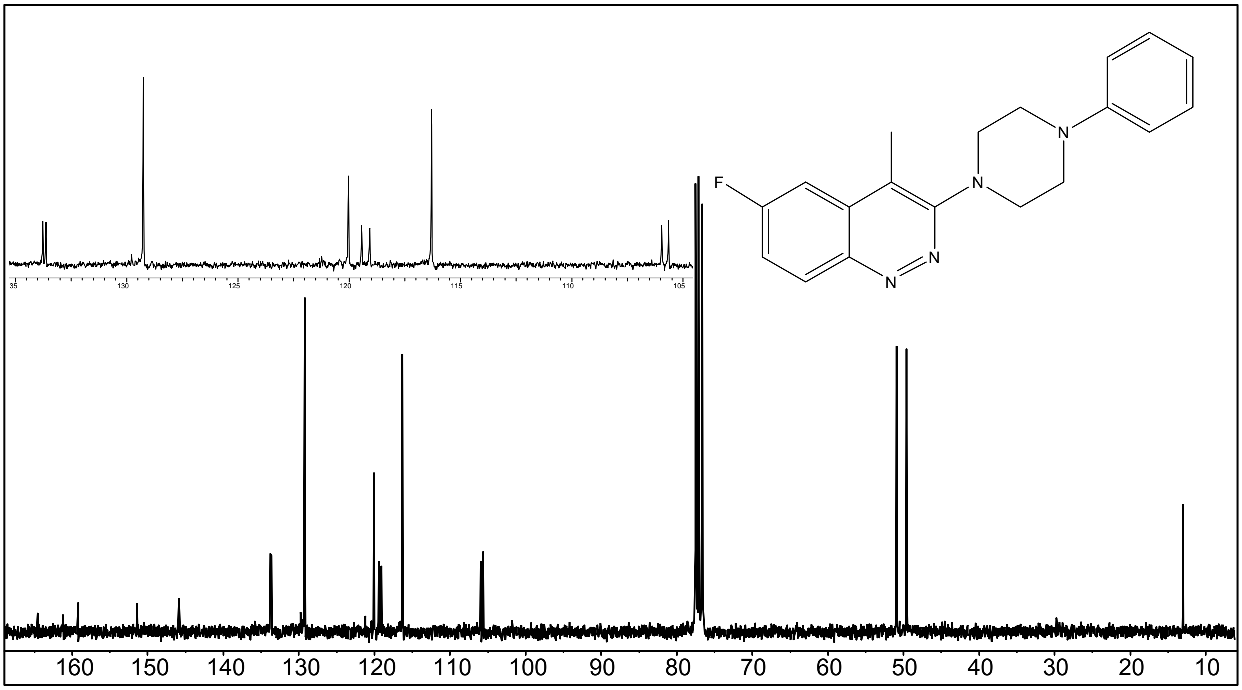


Figure 27: ^{13}C-NMR spectrum of compound [**41b**] ($CDCl_3$)

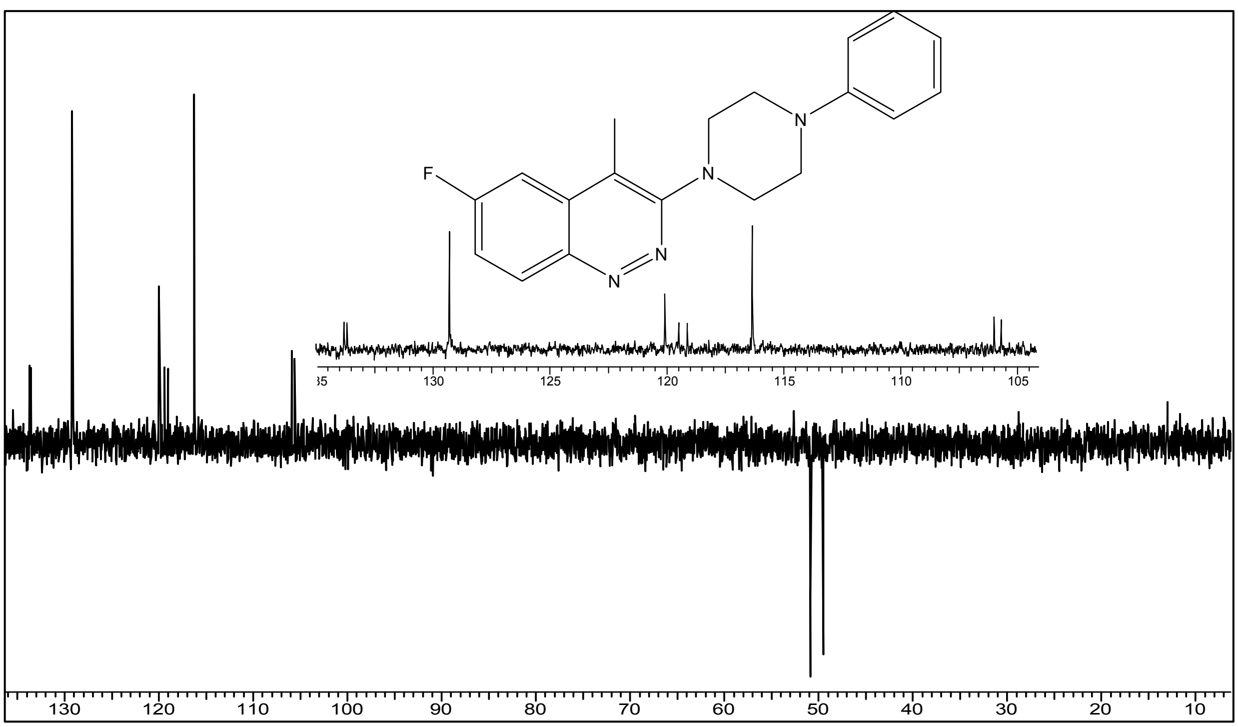


Figure 28: Dept 135 spectrum of compound [**41b**] ($CDCl_3$)

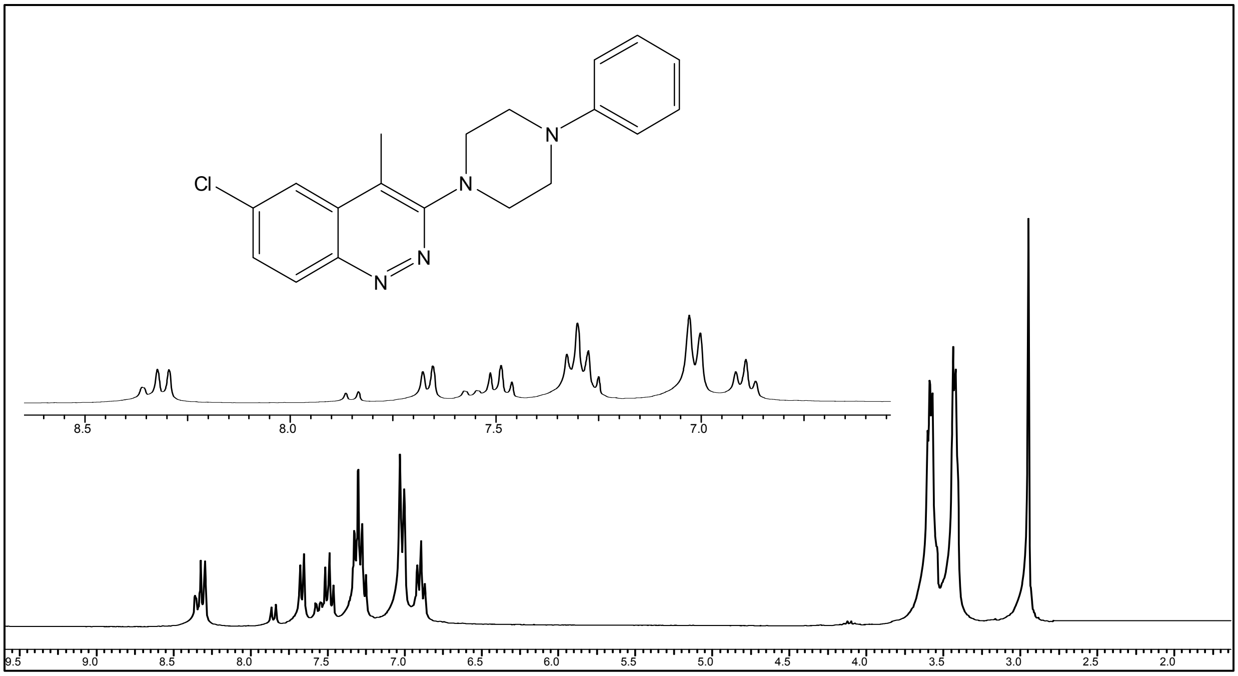


Figure 29: [1]H-NMR spectrum of compound [**41c**] ($CDCl_3$)

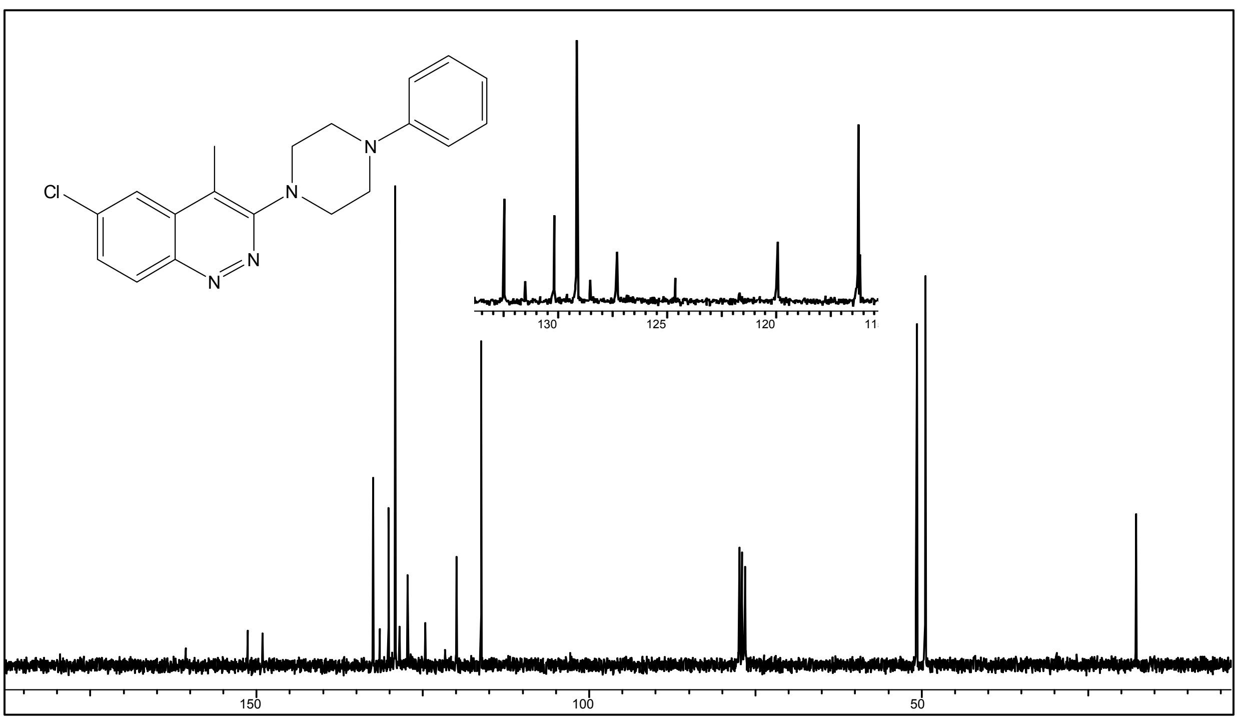


Figure 30: [13]C-NMR spectrum of compound [**41c**] ($CDCl_3$)

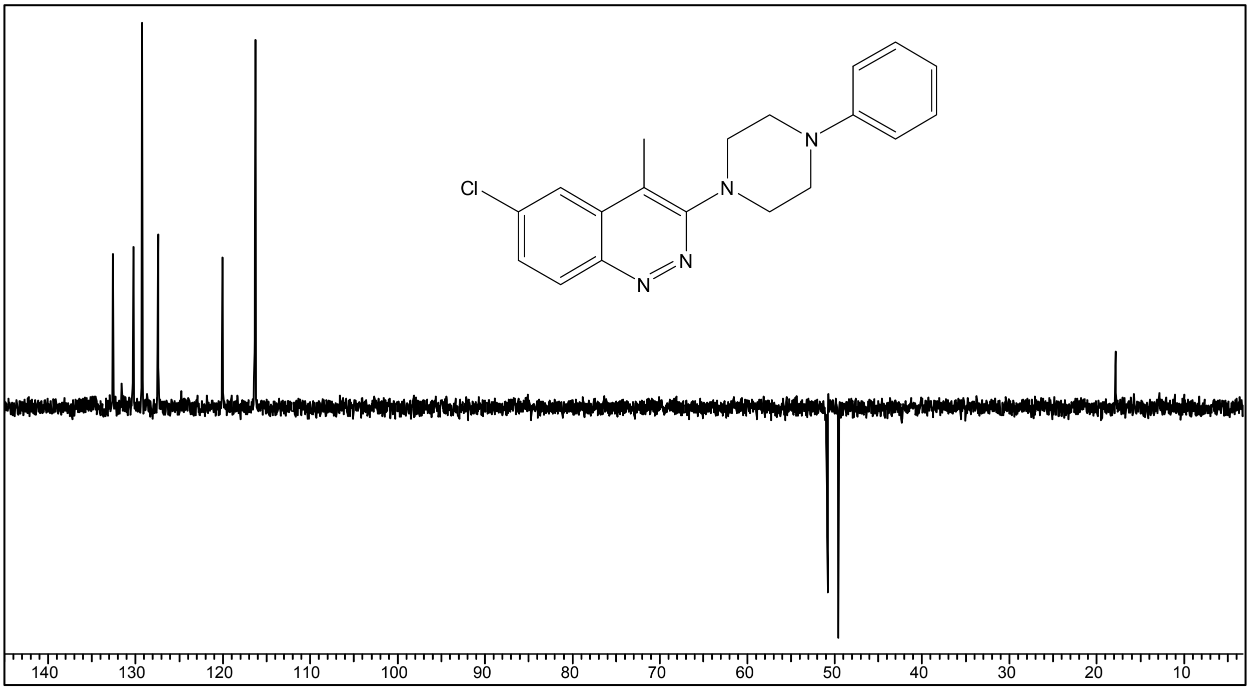


Figure 31: Dept 135 spectrum of compound [**41c**] ($CDCl_3$)

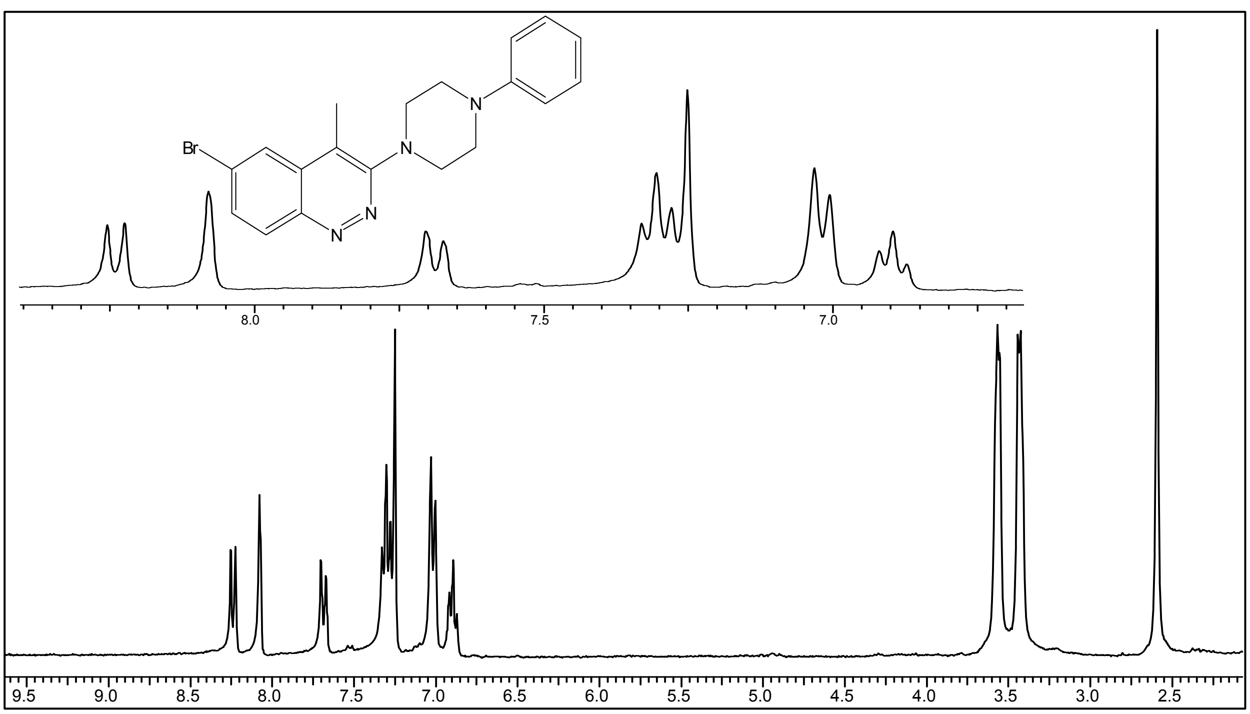


Figure 32: ^{1}H-NMR spectrum of compound [**41d**] ($CDCl_3$)

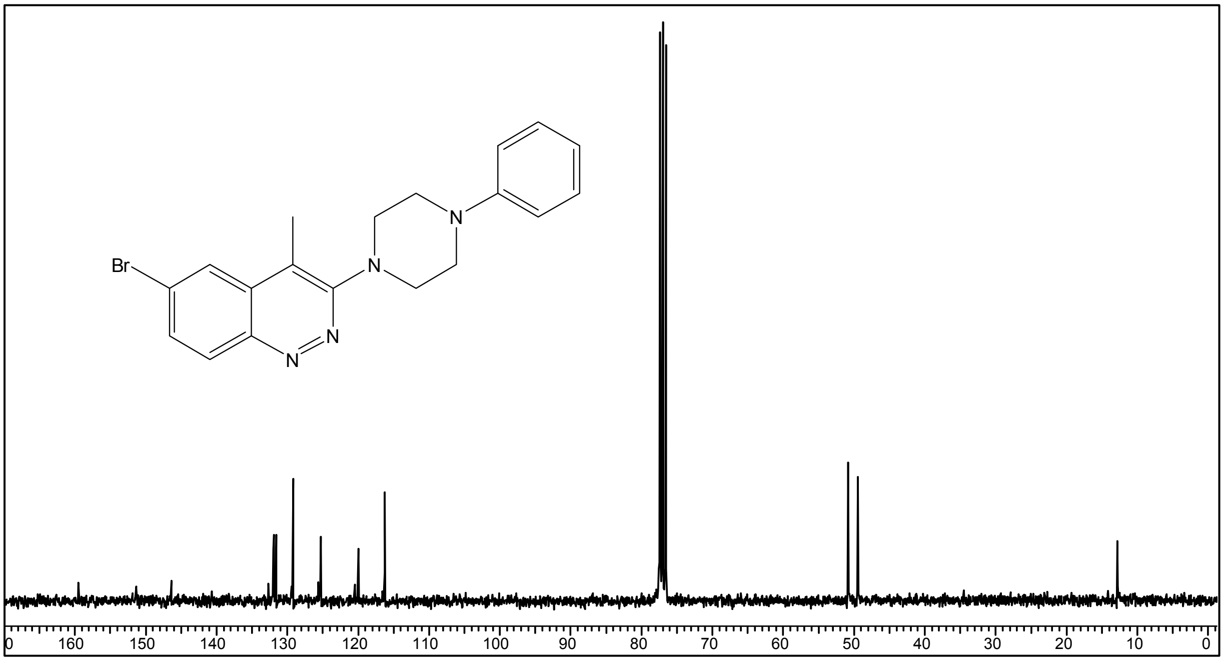


Figure 33: [13]C-NMR spectrum of compound [**41d**] ($CDCl_3$)

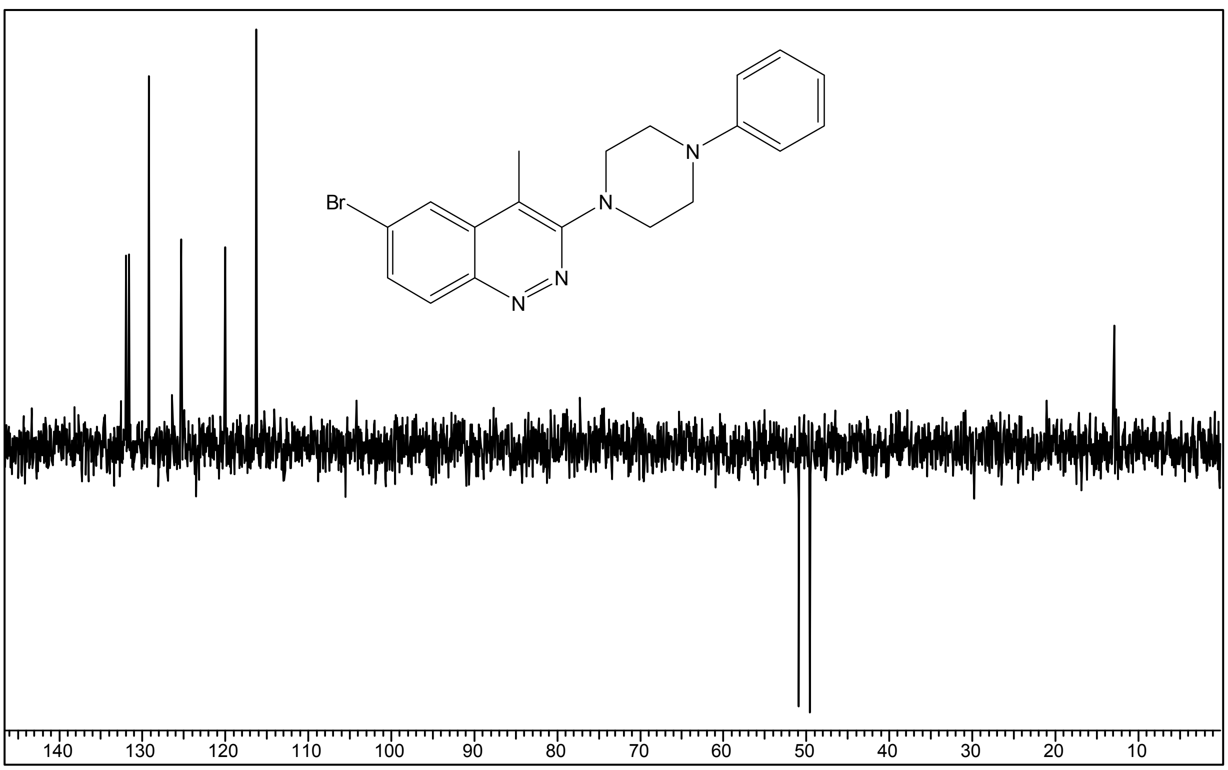


Figure 34: Dept 135 spectrum of compound [**41d**] ($CDCl_3$)

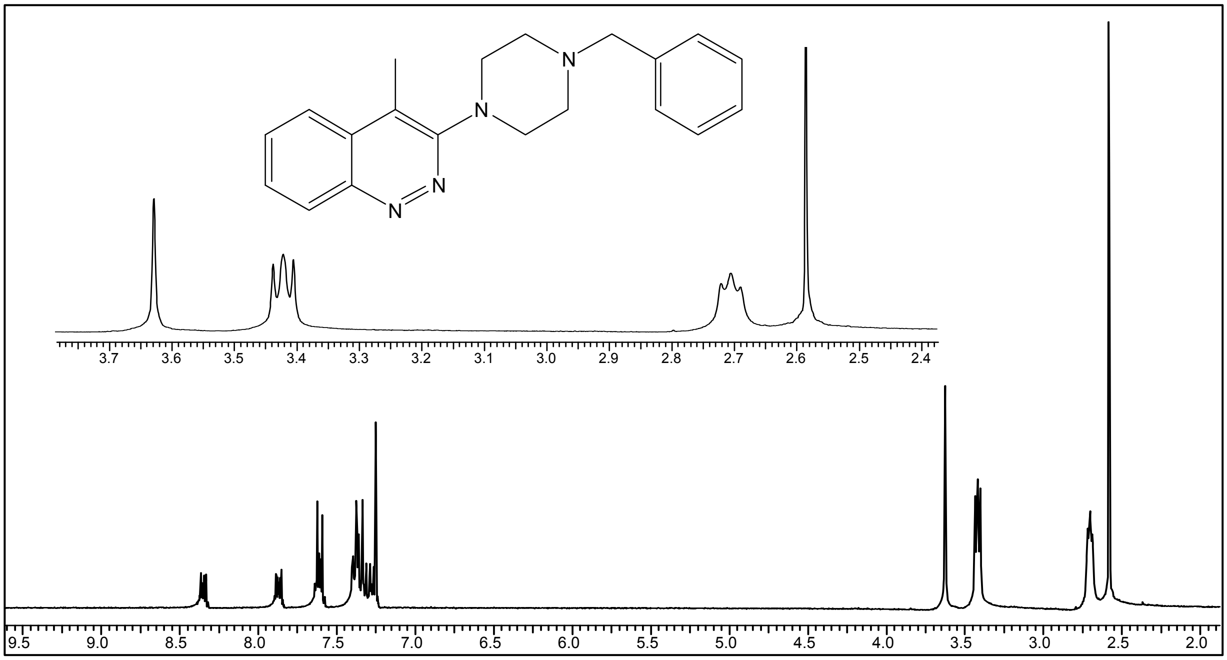


Figure 35: ^{1}H-NMR spectrum of compound [**42a**] ($CDCl_3$)

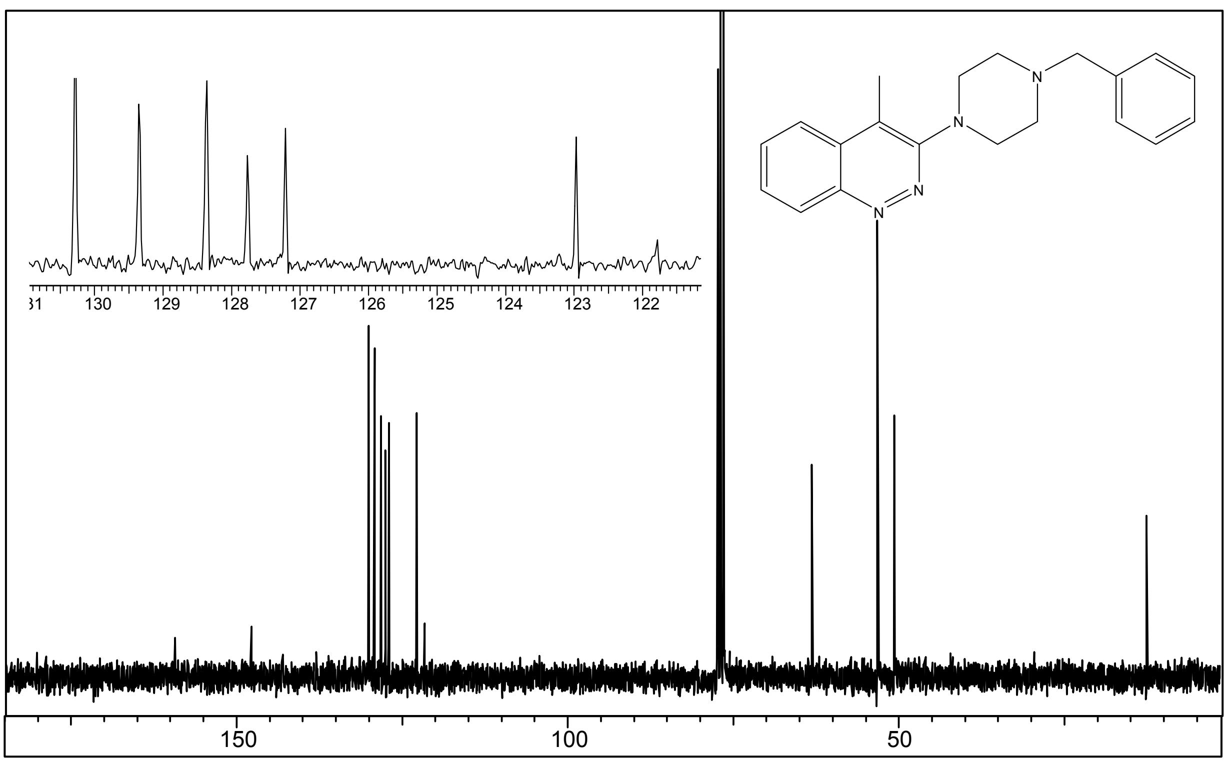


Figure 36: ^{13}C-NMR spectrum of compound [**42a**] ($CDCl_3$)

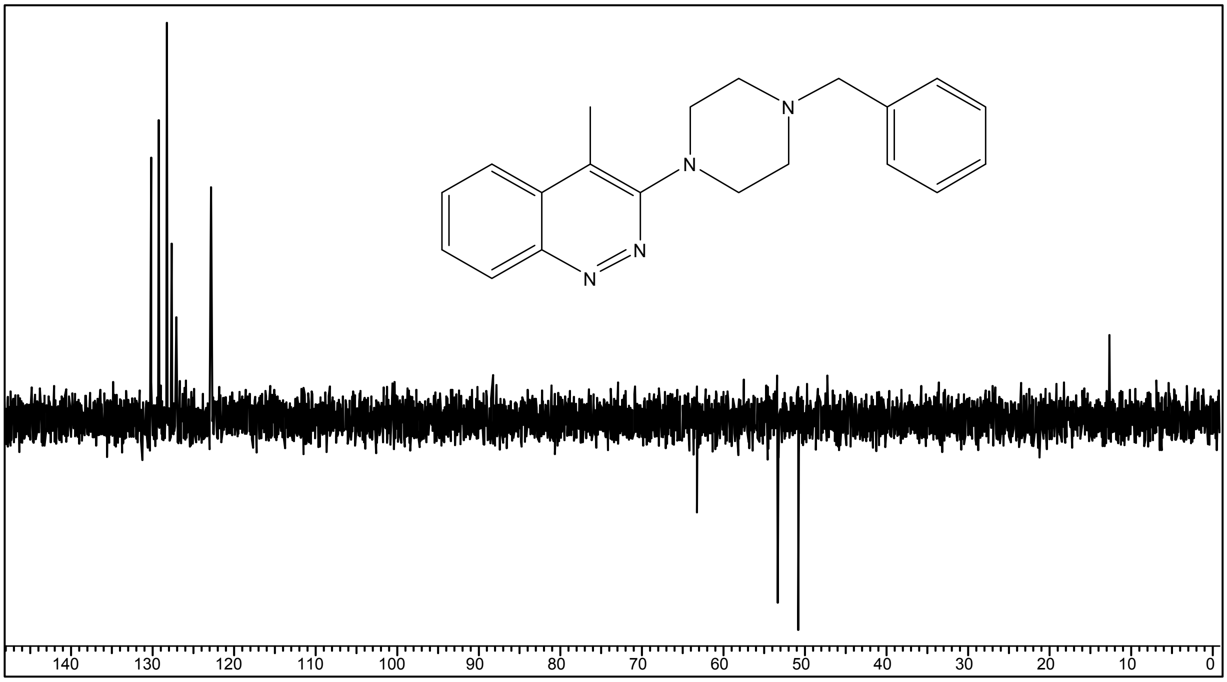


Figure 37: Dept 135 spectrum of compound [**42a**] ($CDCl_3$)

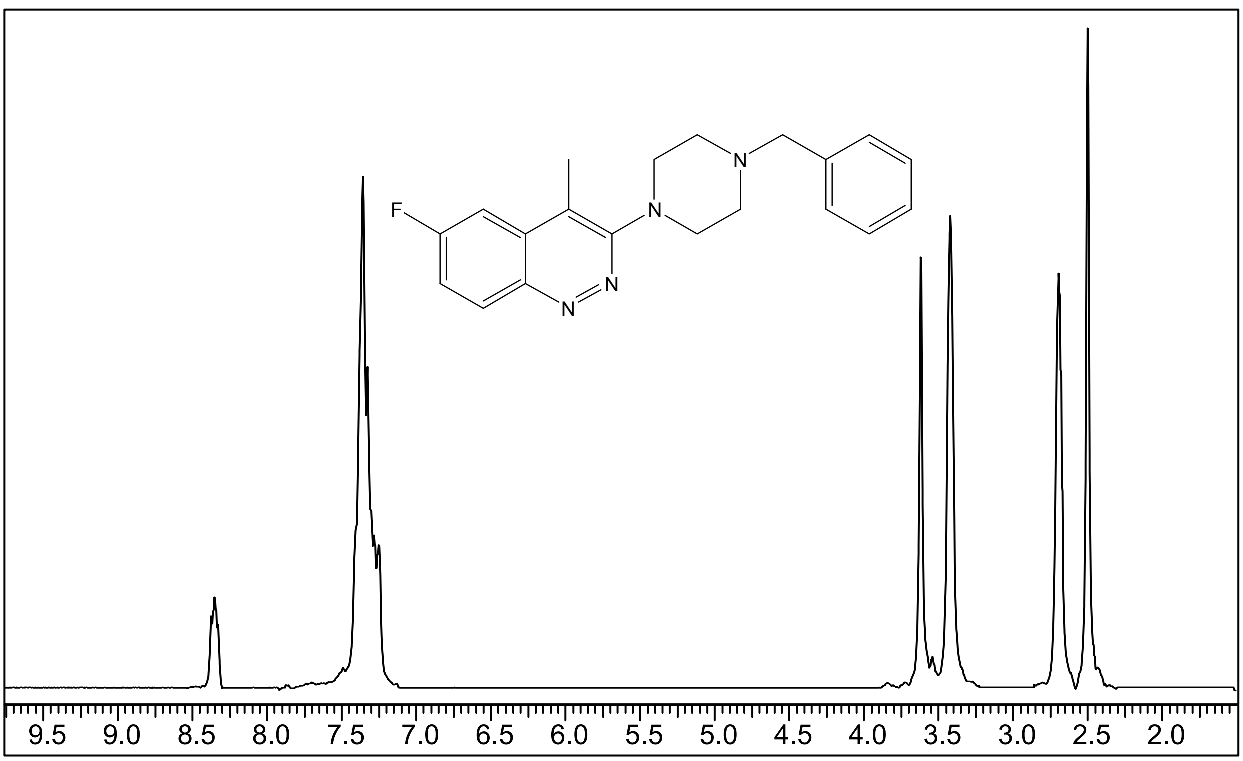


Figure 38: ^{1}H-NMR spectrum of compound [**42b**] ($CDCl_3$)

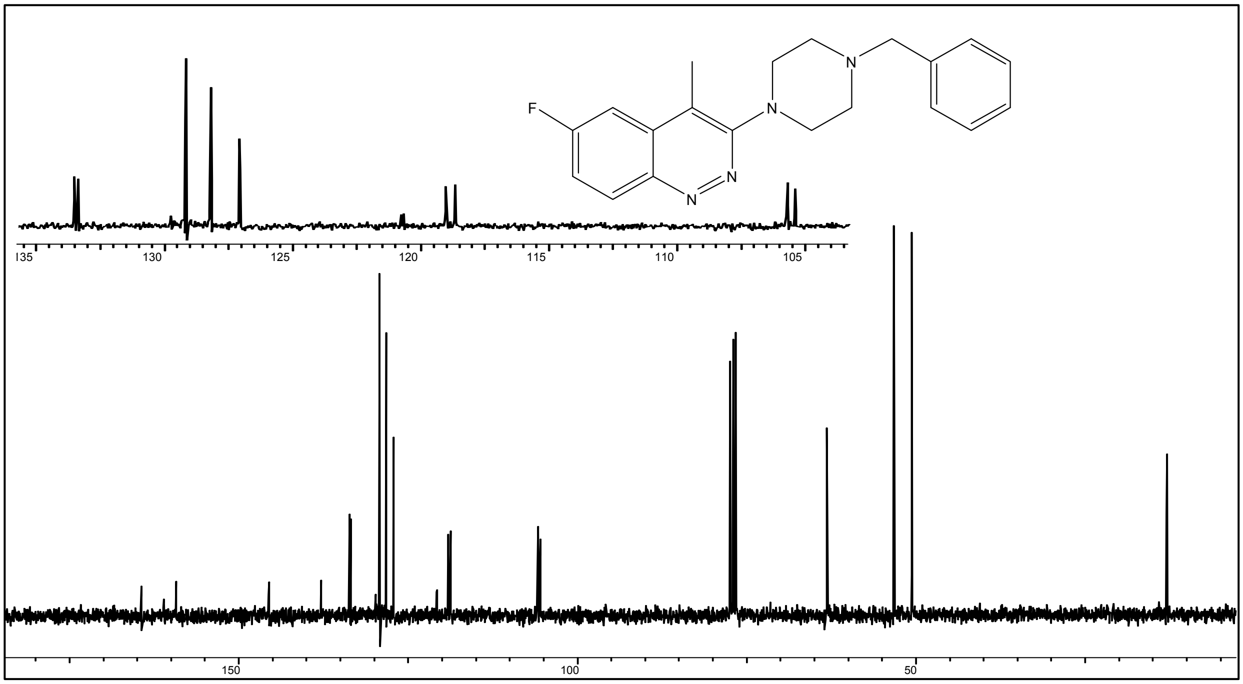


Figure 39: ^{13}C-NMR spectrum of compound [**42b**] ($CDCl_3$)

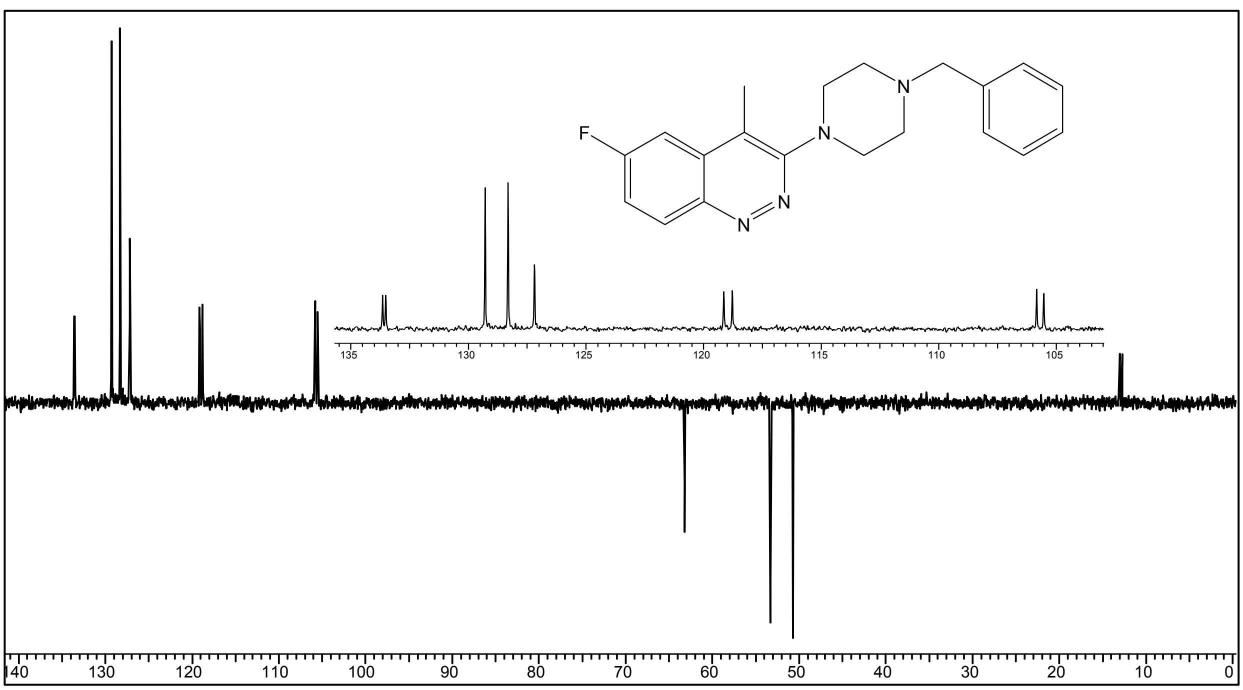


Figure 40: Dept 135 spectrum of compound [**42b**] ($CDCl_3$)

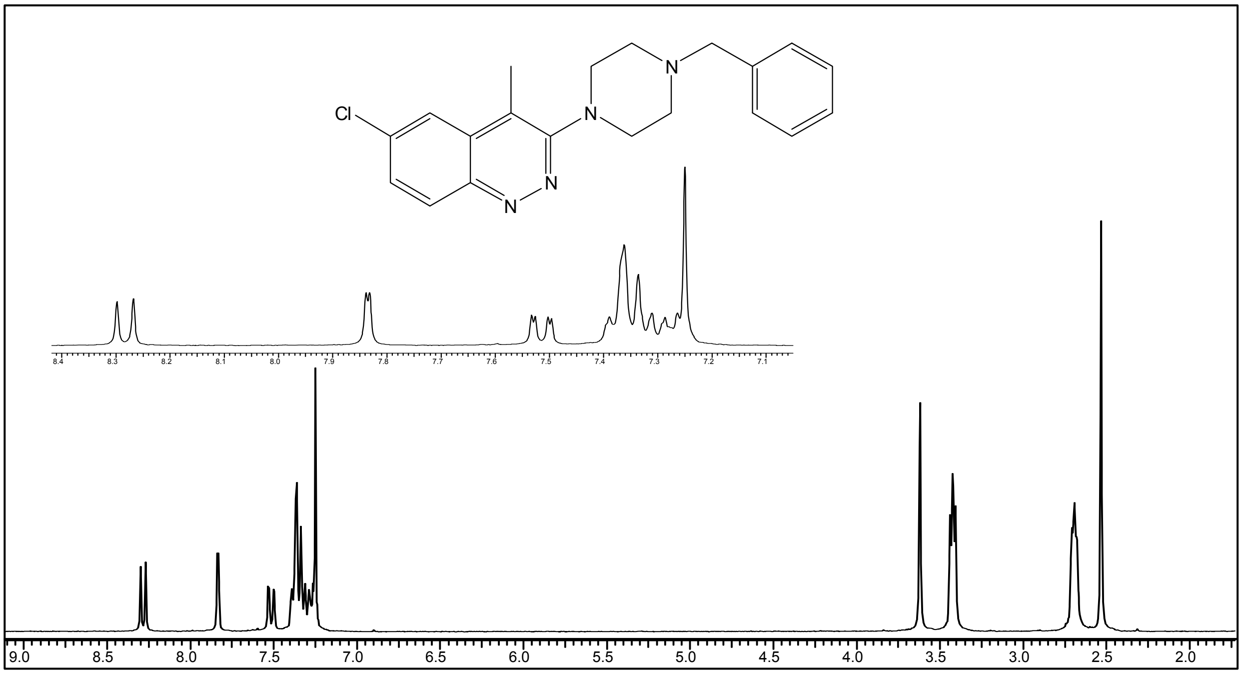


Figure 41: ^{1}H-NMR spectrum of compound [**42c**] ($CDCl_3$)

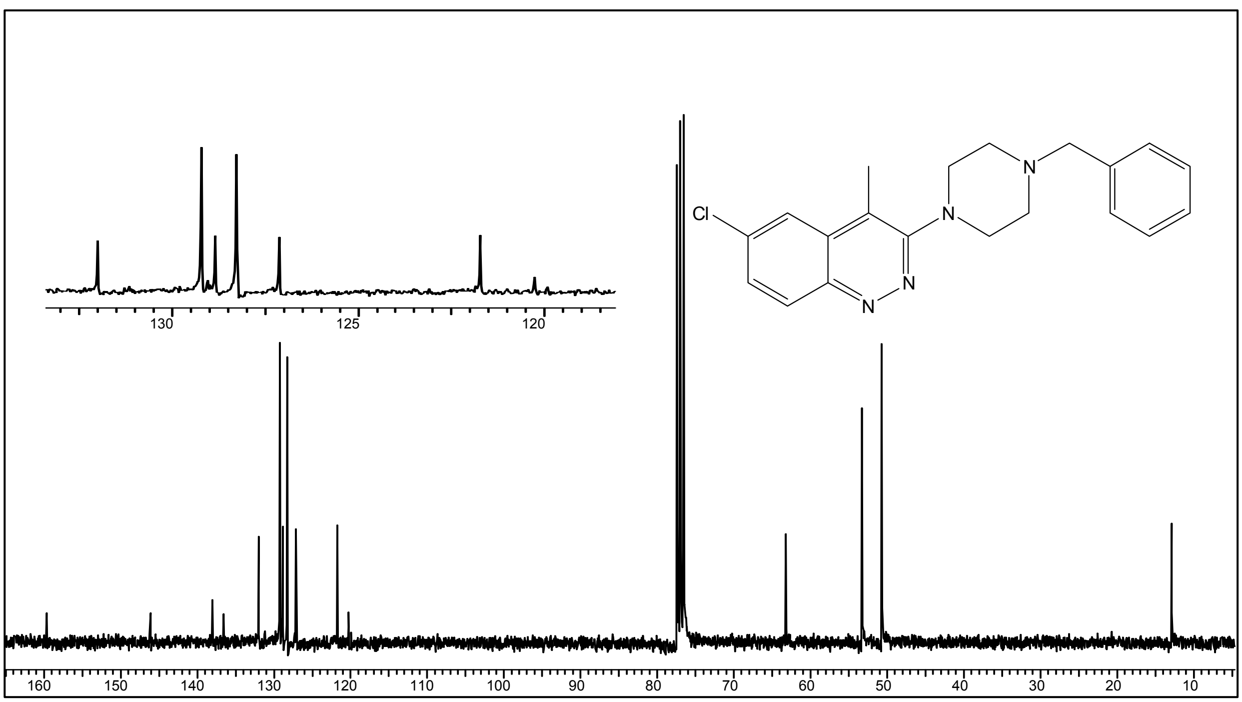


Figure 42: ^{13}C-NMR spectrum of compound [**42c**] ($CDCl_3$)

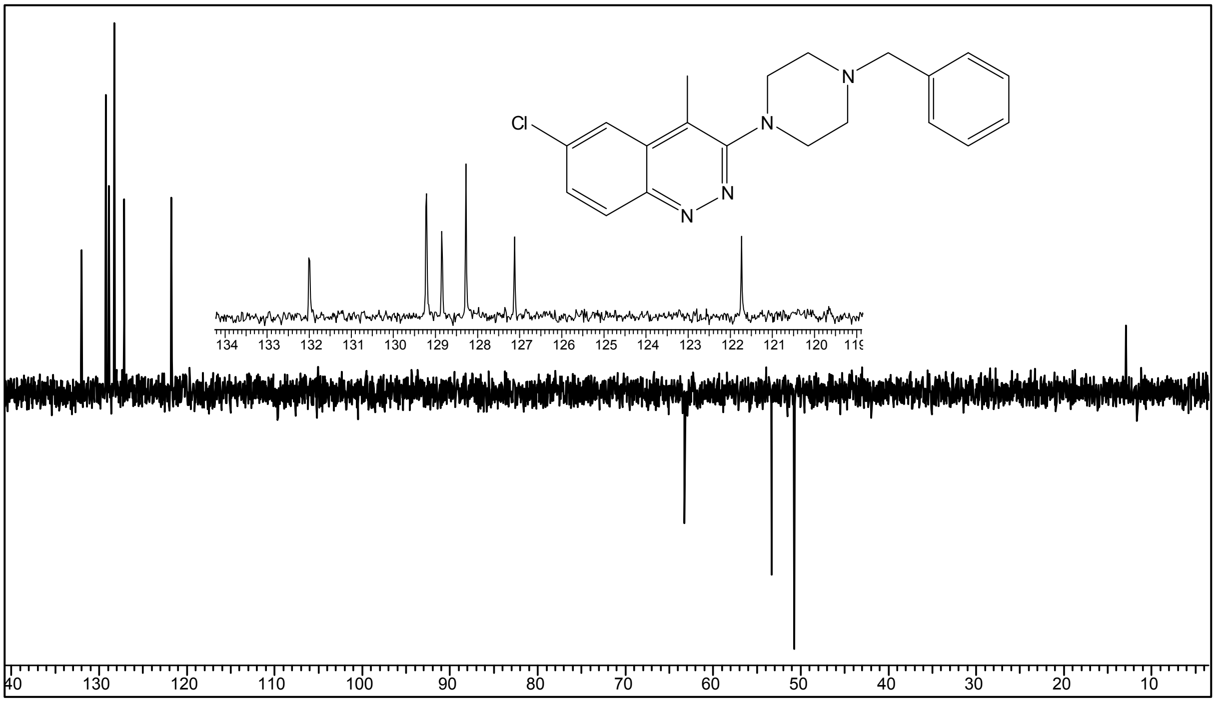


Figure 43: Dept 135 spectrum of compound [**42c**] ($CDCl_3$)

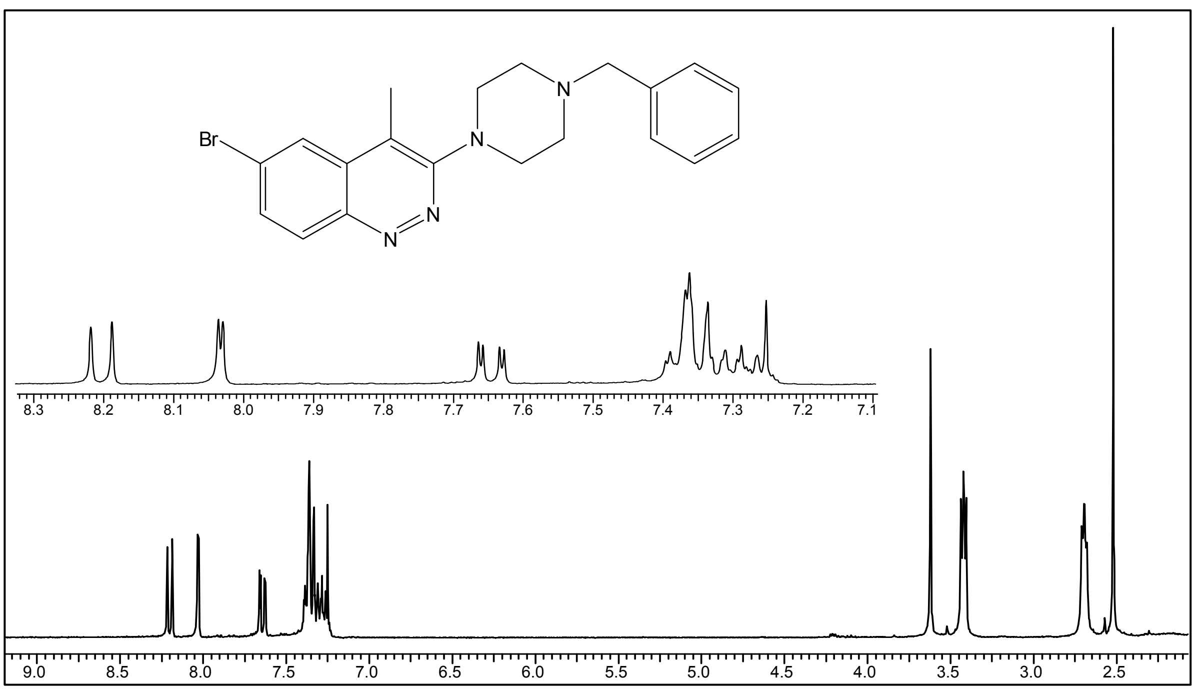


Figure 44: ^{1}H-NMR spectrum of compound [**42d**] ($CDCl_3$)

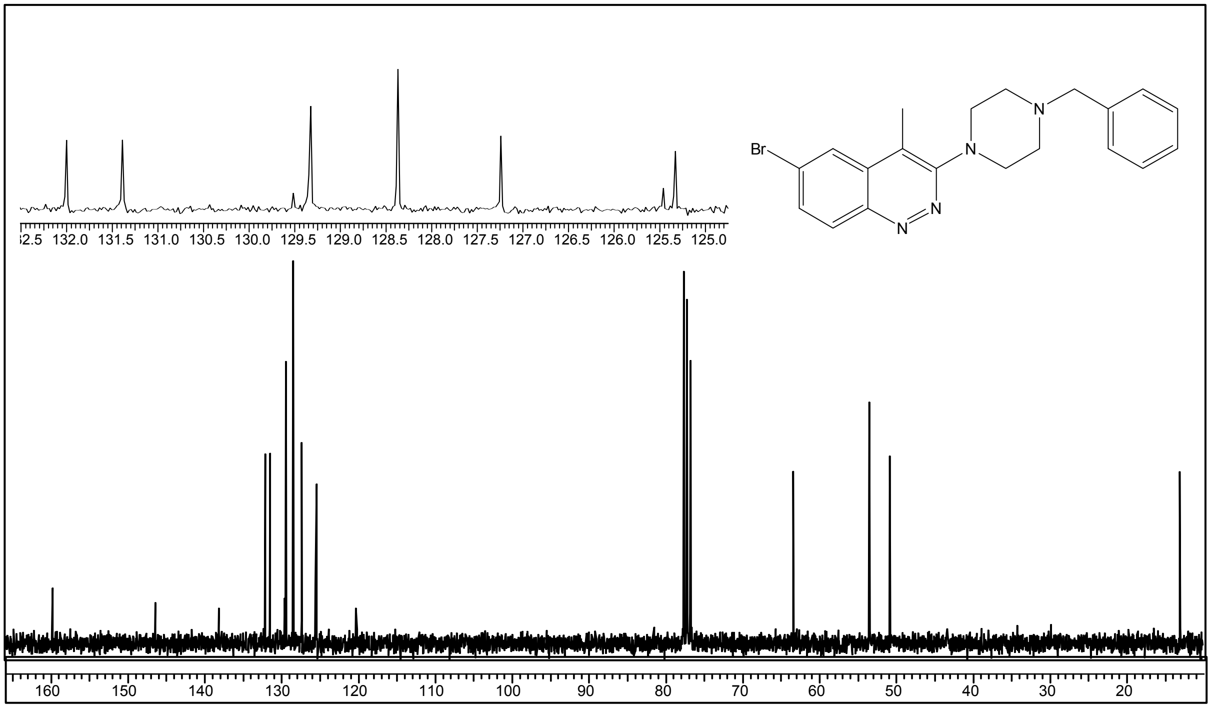


Figure 45: ^{13}C-NMR spectrum of compound [**42d**] ($CDCl_3$)

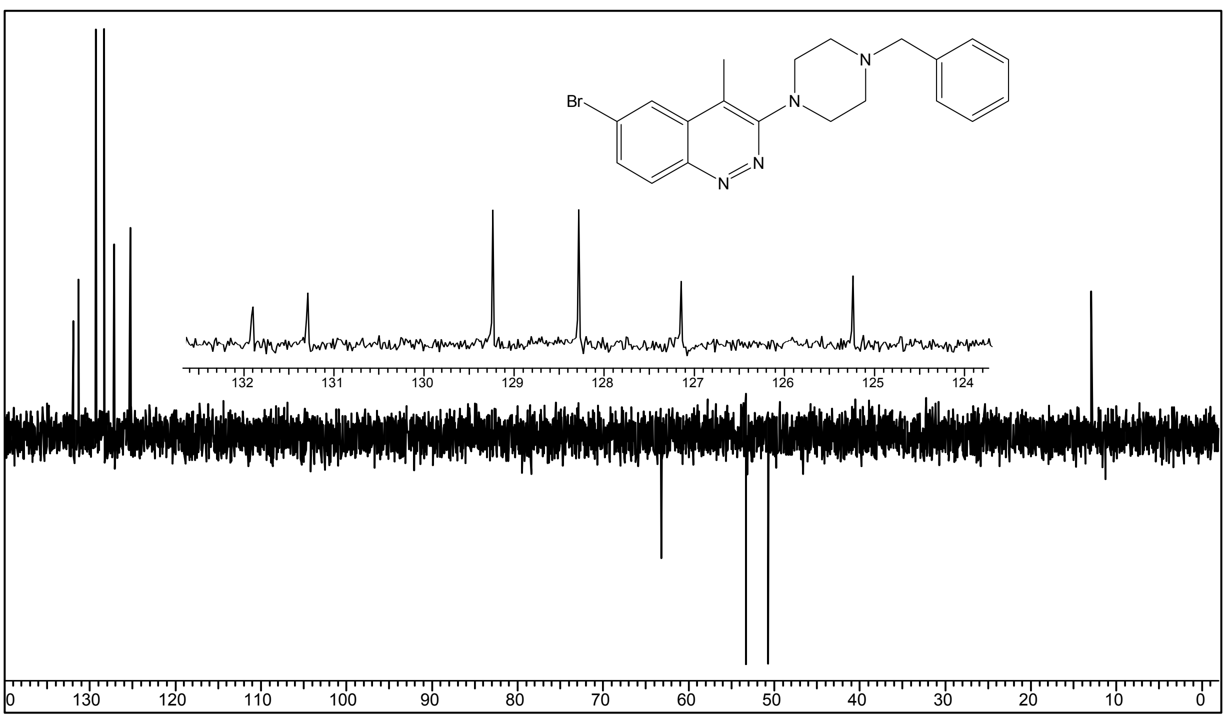


Figure 46: Dept 135 spectrum of compound [**42d**] ($CDCl_3$)



Figure 47: ^{1}H-NMR spectrum of compound [**43a**] ($CDCl_3$)

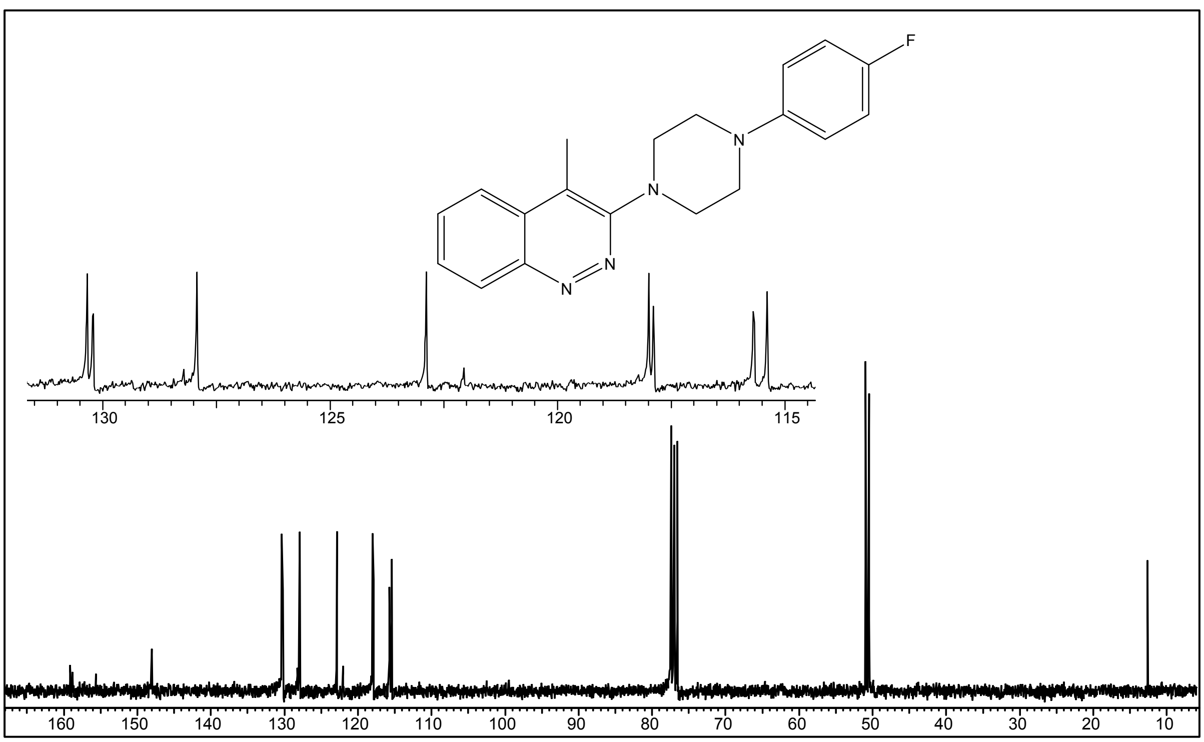


Figure 48: ^{13}C-NMR spectrum of compound [**43a**] ($CDCl_3$)

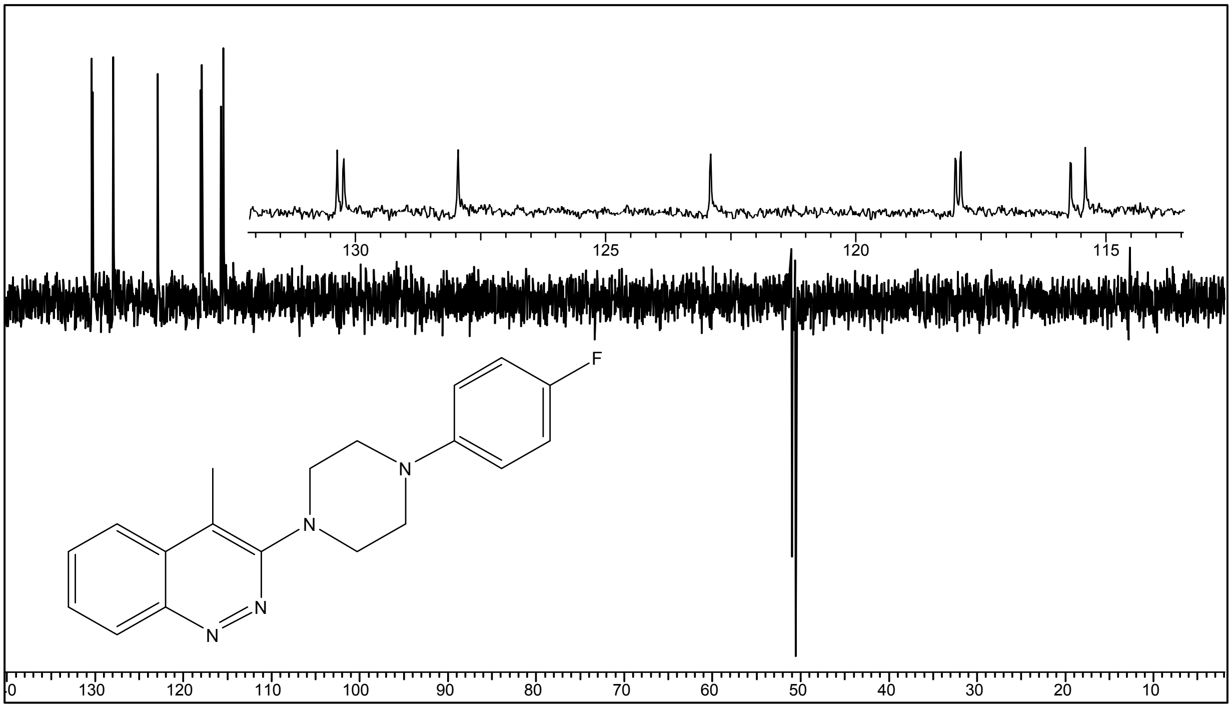


Figure 49: Dept 135 spectrum of compound [**43a**] ($CDCl_3$)

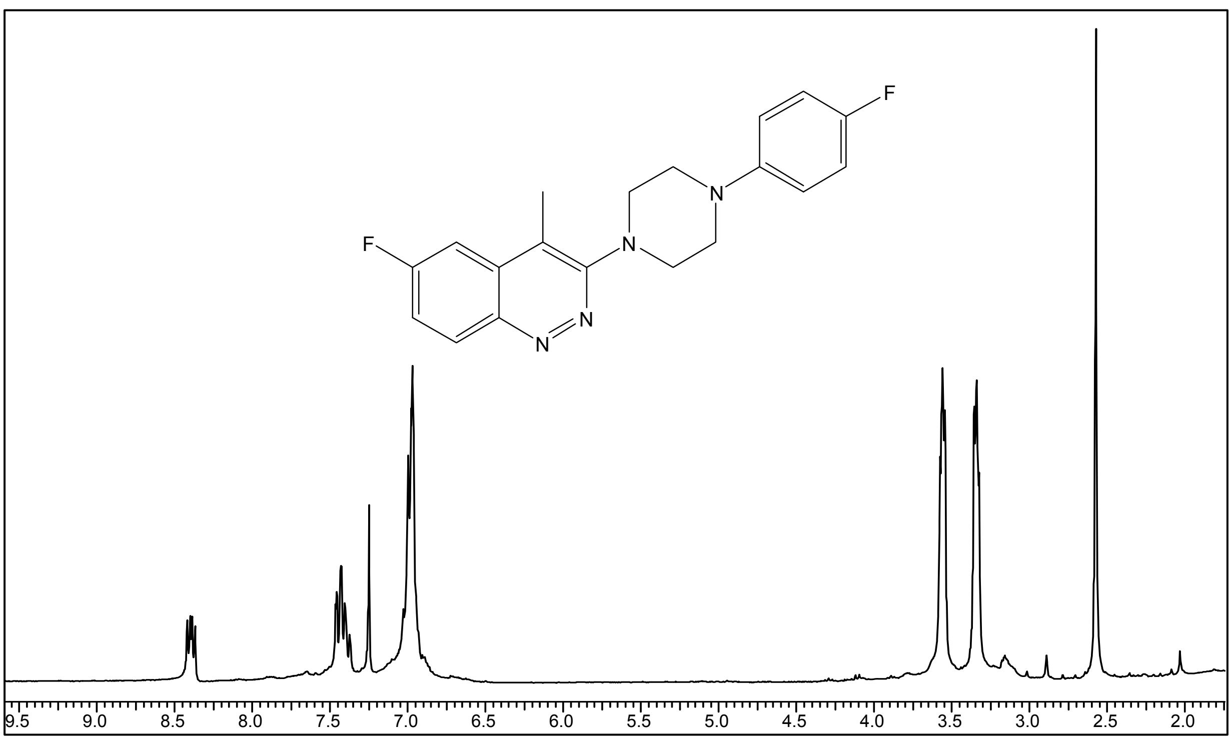


Figure 50: ^{1}H-NMR spectrum of compound [**43b**] ($CDCl_3$)

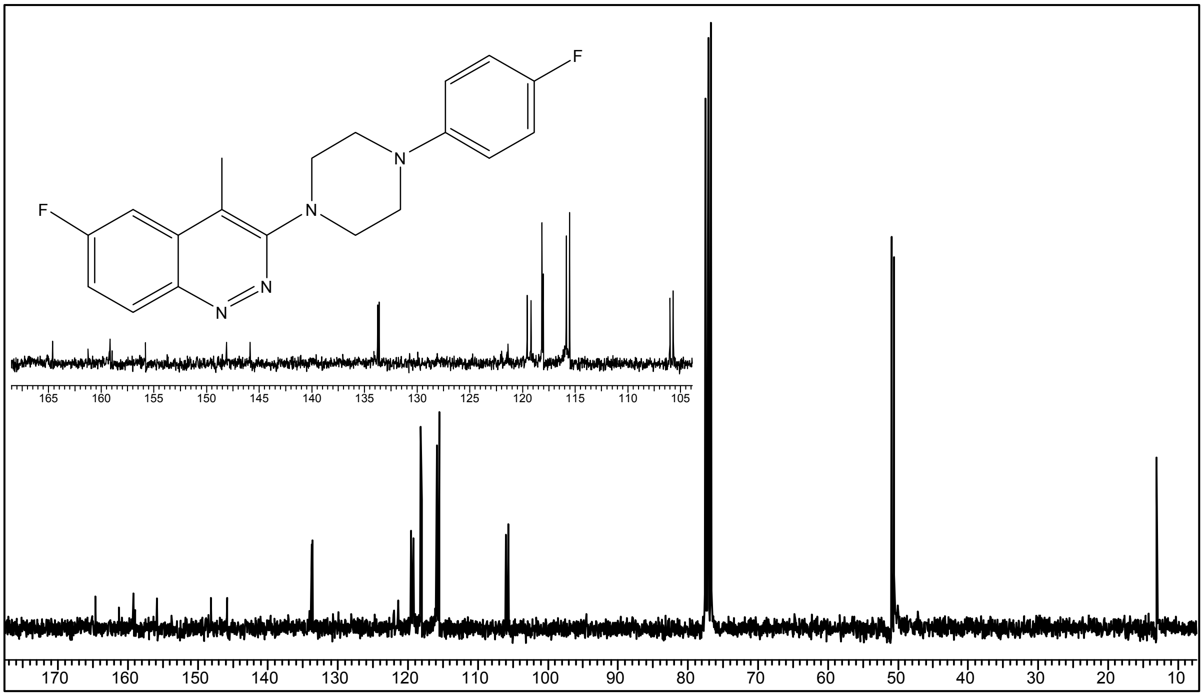


Figure 51: ^{13}C-NMR spectrum of compound [**43b**] ($CDCl_3$)

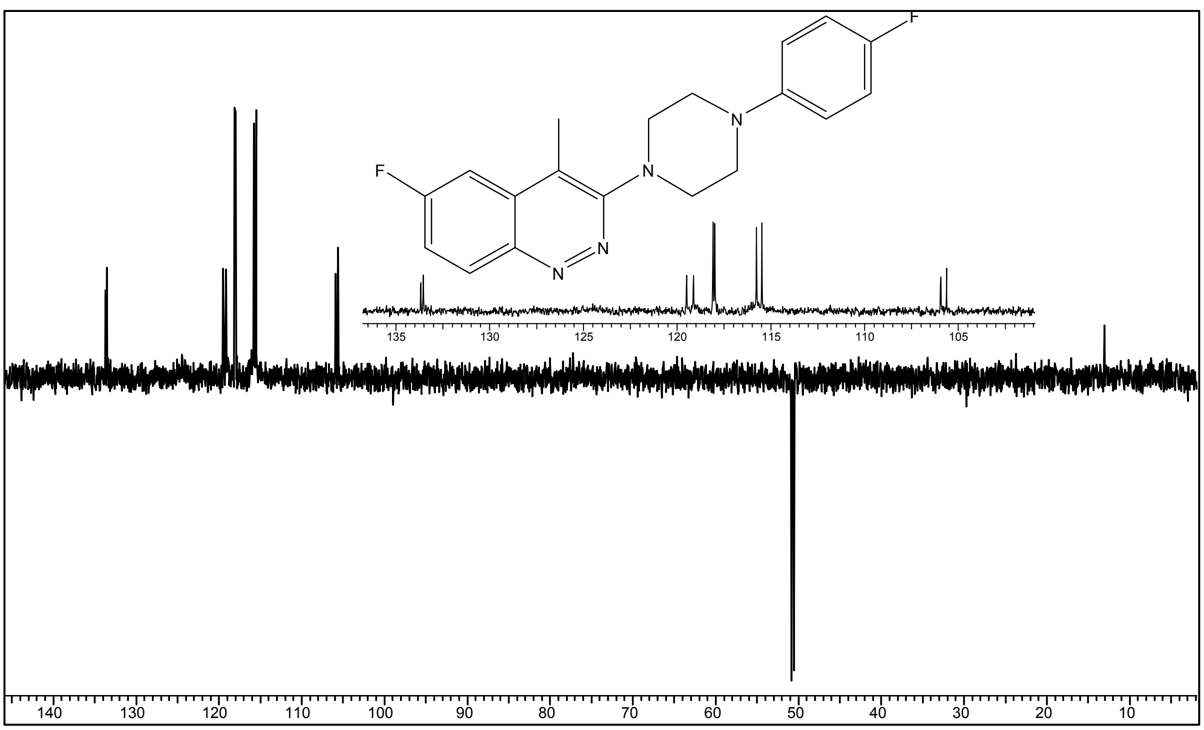


Figure 52: Dept 135 spectrum of compound [**43b**] ($CDCl_3$)

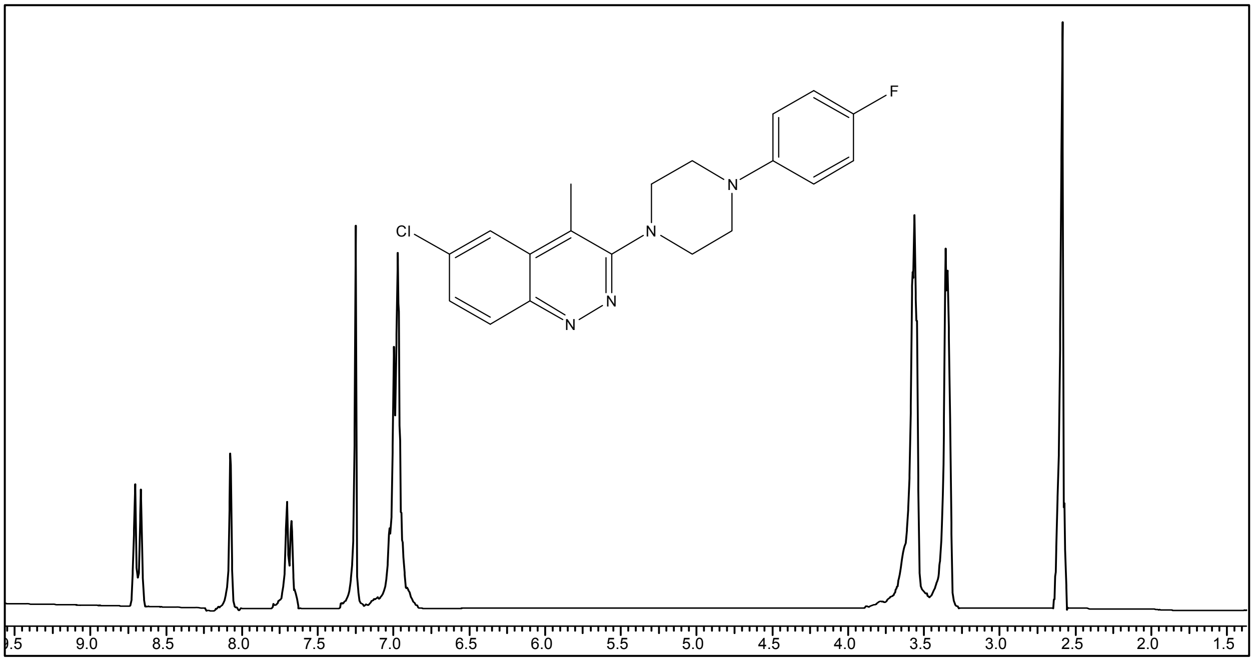


Figure 53: ^{1}H-NMR spectrum of compound [**43c**] ($CDCl_3$)

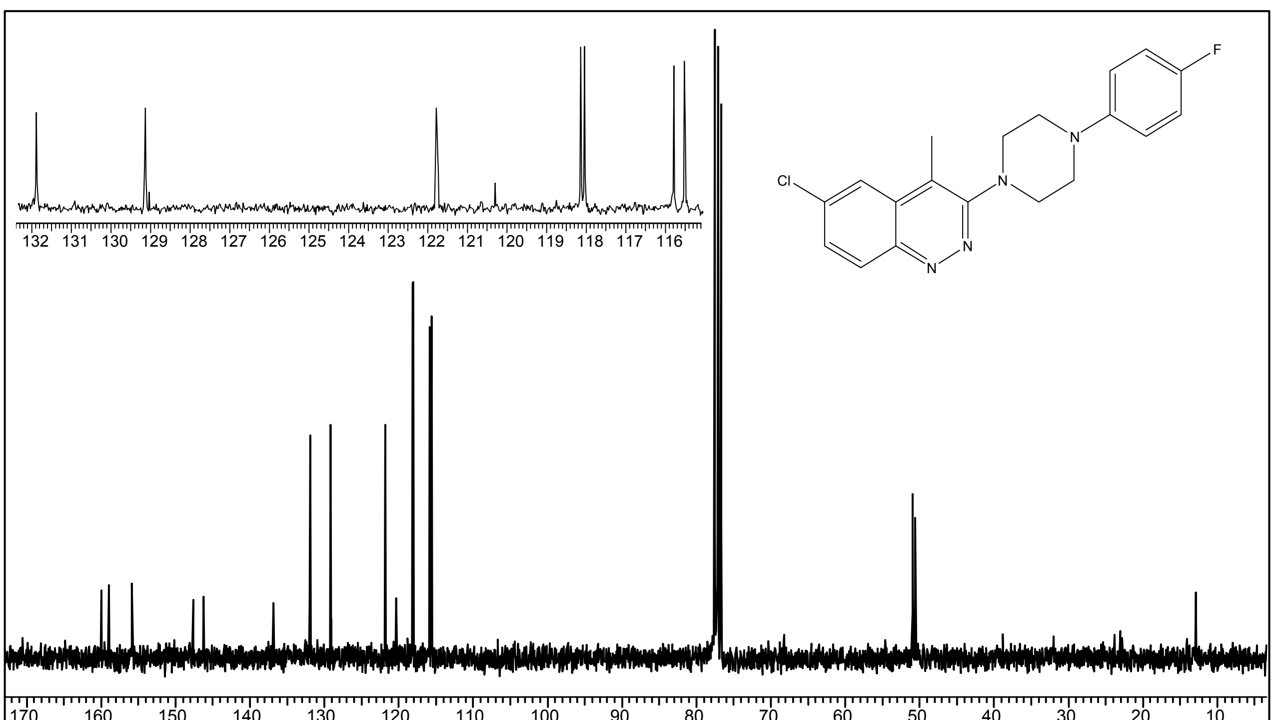


Figure 54: ^{13}C-NMR spectrum of compound [**43c**] ($CDCl_3$)

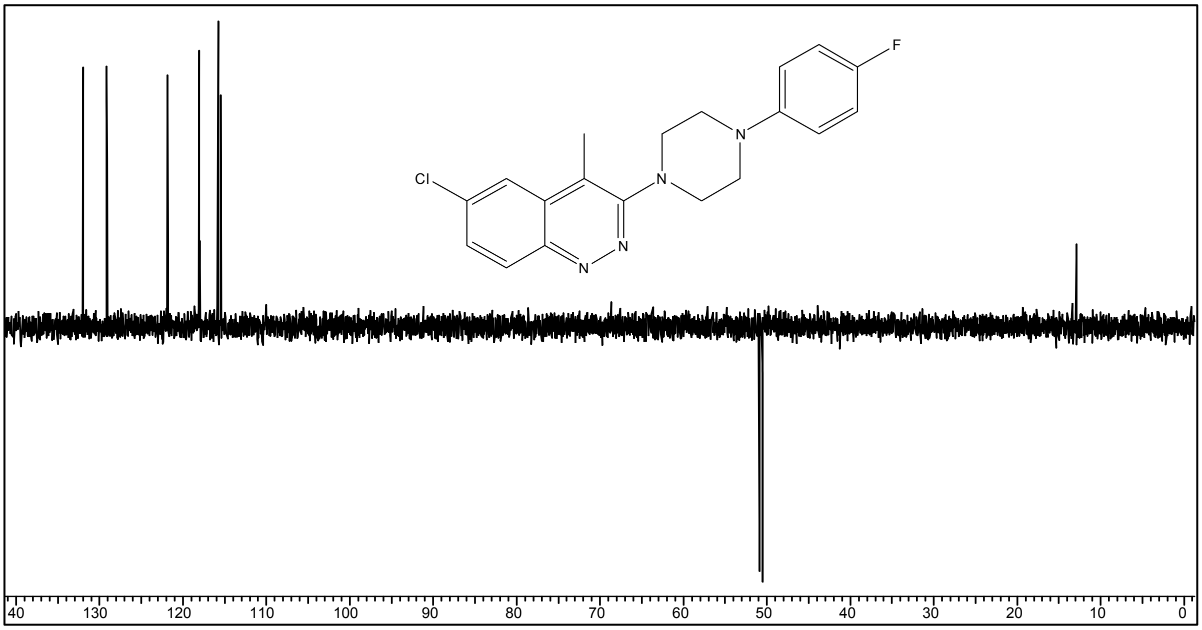


Figure 55: Dept 135 spectrum of compound [**43c**] ($CDCl_3$)

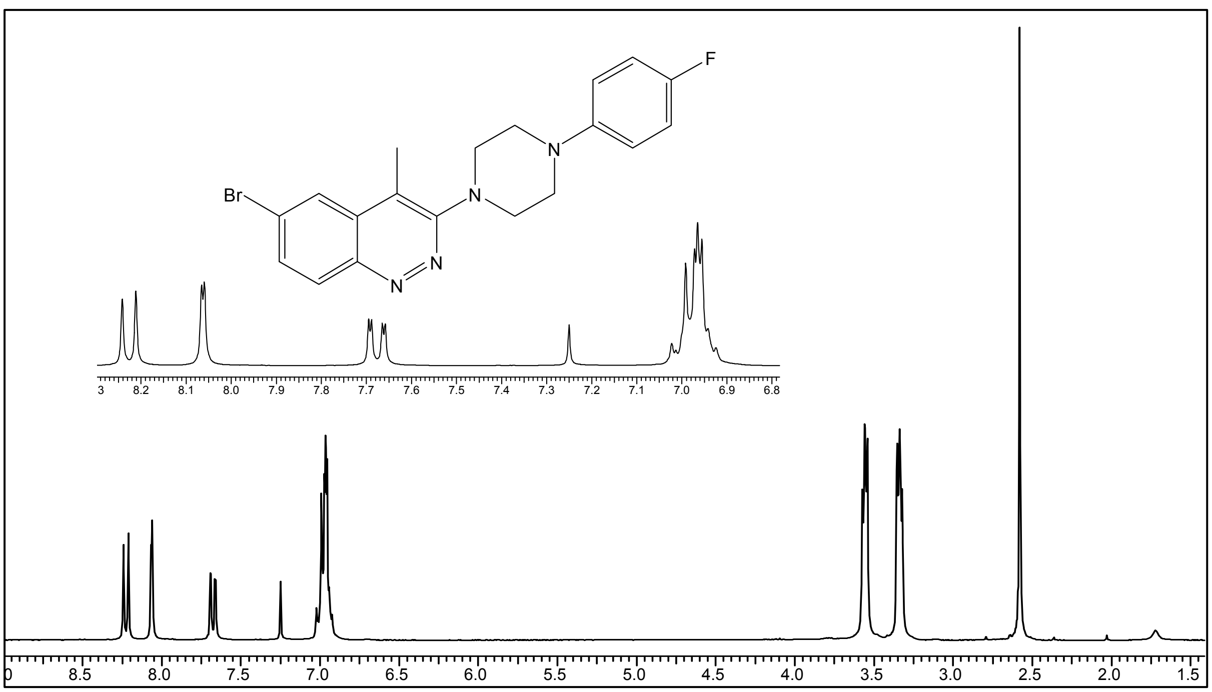


Figure 56: ^{1}H-NMR spectrum of compound [**43d**] ($CDCl_3$)

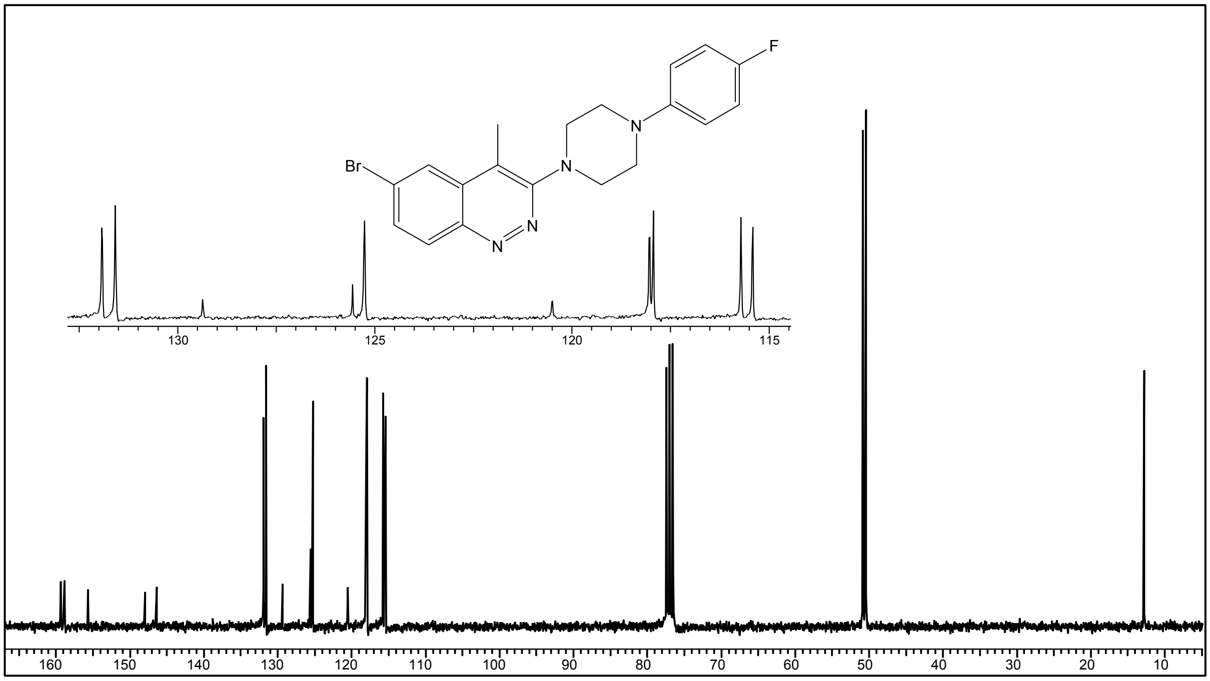


Figure 57: ^{13}C-NMR spectrum of compound [**43d**] ($CDCl_3$)

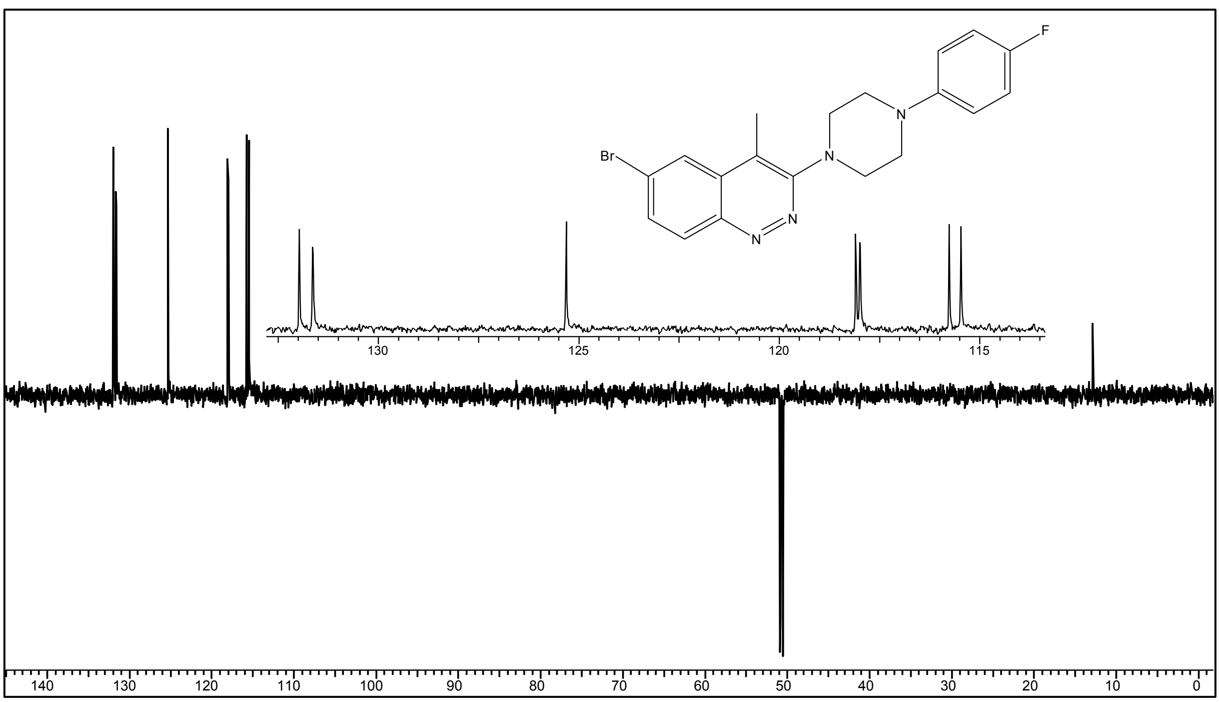


Figure 58: Dept 135 spectrum of compound [**43d**] ($CDCl_3$)

تحضير و تشخيص و دراسة التأثيرات البيولوجية لبعض مشتقات 3-(ببرازين-1-يل) سينولين

إعداد
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الملخص

حضر عدد من مشتقات 6-معوض-ميثيل-3-(4-اريل-1-ببرازينيل)سينولين الجديدة **(41-43)** كمواد يمكن أن تستخدم مضادات للبكتيريا و الفطريات، عن طريق التحولق الضمني لمشتقات ببيرازينيل ايميدرازون **(38-40)** بواسطة متعدد حمض الفوسفوريك.

و تم تحضير مركبات البيبرازينيل ايميدرازون عن طريق تفاعل مركبات كلورايد الهيدرازونيل مع بعض مشتقات البيبرازين في وسط قاعدي. وقد تم تشخيص هذه المركبات الجديدة وموافقتها للتراكيب المقترحة باستخدام بعض التقنيات الطيفية كطيف الرنين النووي المغناطيسي للهيدروجين والكربون-13 و مطياف الكتلة.